C. elegans Topic Meeting: Neuronal Development, Synaptic Function and Behavior

July 24-27, 2022
University of Vienna

Program and Abstract Book

Table of content

Organizers

Scientific Organizers

Henrik Bringmann, Technical University Dresden, Germany.
Luisa Cochella, Johns Hopkins University School of Medicine, Maryland, USA.
Alexander Gottschalk, University of Frankfurt, Germany.
Manuel Zimmer, University of Vienna, Austria.

Scientific Committee
Nuria Flames, Instituto de Biomedicina de Valencia, Valencia, Spain.
Anne Hart, Brown University, Boston, USA.
Max Heiman, Harvard University, Boston, USA.
Massimo Hilliard, Queensland Brain Institute, Brisbane, Australia.
Kotaro Kimura, Nagoya City University, Japan.
David Miller, Vanderbilt University, Nashville, USA.
Georgia Rapti, European Molecular Biology Laboratory, Heidelberg, Germany.
Janet Richmond, University of Illinois, Chicago, USA.
Monika Scholz, Caesar Institute, Bonn, Germany.
Young-Jai You, University of Nagoya, Japan.
Yun Zhang, Harvard University, Boston, USA.

Program

DAY 1, 24th July 2022

12:00 - 17:00: Registration and set up of posters (Session I, Posters 1-80)

17:00 - 17:10: Opening of the meeting and general information

Plenary Session 1: Technological Advances and Community Resources
Session Chair: David Miller

17:10 - 17:40: Keynote lecture 1
Mei Zhen
Developmental strategies to maintain locomotory output

17:40 - 18:40: Four short talks from selected abstracts
1) Dennis Vettkötter: Rapid and reversible optogenetic silencing of synaptic transmission by clustering of synaptic vesicles
2) Jan Watteyne: Visualizing neuropeptide GPCR activation in *C. elegans* using PepSee
3) Kieran Baxter: Using genetic code expansion to develop a photo-activatable FLP recombinase.
4) Elsa Bonnard: Automatically tracking feeding behavior in populations of foraging *C. elegans*

19:00 - 20:00: Dinner (catering on site)

20:00 - 23:00: Poster session I, Posters 1-80 (drinks + snacks available) (catering on site)

DAY 2, 25th July 2022
Plenary Session 2: Neurodevelopment
Session Chair: Georgia Rapti

09:00 set up of posters Session II

09:00 - 09:30: Keynote lecture 2
Shai Shaham
Astroglial control of *C. elegans* behavior

09:30 - 10:30: Four short talks from selected abstracts
1) Leo Tsz-Ho Tang: Insulin-like signaling regulates left/right asymmetric synaptic connectivity
2) Raphael Dima: Syndecan, netrin, guidance receptors and Rho-family GTPases cooperate to regulate the number of neurites/cellular extensions in neurons and other polarized cells
3) Caitlin Taylor: HDAC inhibition combats neurodevelopmental trafficking stressors
4) Dhanya Cheerambathur: Repurposing the Chromosome-Microtubule Coupling Machinery for Dendritic Branching.

10:30 - 11:00: Coffee Break (catering on site)

Session Chair: Gert Jansen

11:00 - 12:30: Six Short talks from selected abstracts
1) Hyunsoo Yim: The mind of a dauer: EM reconstruction for the dauer connectome
2) Sneha Hegde: Axonal mitochondria regulate gentle touch response through control of actin dynamics
3) HaoSheng Sun: Temporal Maturation of the C. elegans Post-Embryonic Nervous System
4) Karen Juanez: ER network stability promotes organized microtubule disassembly during Compartmentalized Cell Elimination
5) En-Ni Chang: Neuronal Mitochondrial Dynamics Coordinate Systemic Mitochondrial Morphology through Tyramine Signaling
6) Shay Stern: Early-life experience reorganizes neuromodulatory regulation of stage-specific behavioral patterns and individuality types during development

12:30 - 14:00: Lunch (catering on site)

Plenary Session 3: Neural Circuits and Behavior
Session Chair: Saul Kato

14:00 - 14:30: Keynote lecture 3
Bill Schafer
The neuropeptidergic connectome of *C. elegans*

14:30 - 15:30: Four short talks from selected abstracts
1) Sophie Dvali: Mapping the Functional Connectome in *C. elegans*
2) Tosif Ahamed: A circuit for head-body coordination during forward locomotion

3) Lee Tongyoung: FLP-17 neuropeptide and its cognate receptor EGL-6 regulate a novel *C. elegans* oviposition behavior that increases reproductive fitness
4) Raymond Dunn: Closed-loop interrogation of whole-brain dynamics for causal analysis of neural network activity and behavior

15:30 - 16:00: Coffee Break (catering on site)

Session Chair: Arantza Barrios

16:00 - 17:30: Six Short talks from selected abstracts
1) Jungsoo Kim: Internal state modulates brain-wide representations of behavior in *C. elegans*
2) Yuuki Onishi: Neuropeptide NLP-47 and its receptor GNRR-1 promote forgetting of olfactory memory in *C. elegans*
3) Netta Cohen: How worms explore 3D space
4) Emmanuel Medrano: Mechanosensory feedback regulates egg-laying circuit activity and behavior of *C. elegans*
5) Inka Busack: Overactivation of a sleep-active neuron decouples survival from the need to sleep
6) Eduard Bokman: Encoding principles of a compact sensory system

17:30 - 18:00: Keynote lecture 4
Yuichi Iino
Molecules and neural network underlying salt preference

18:30 - 20:00: Dinner (catering on site)
20:00 - 23:00: Poster Session II, Posters 81-159 (drinks + snacks available) (catering on site)

DAY 3, 26th July 2022

Plenary Session 4: Synaptic functions
Session Chair: Bérangère Pinan-Lucarré

09:00 - 09:30: **The EMBO Keynote lecture**
Jean-Louis Bessereau
The ins and outs of synaptic domain specification

09:30 - 10:30: Four short talks from selected abstracts
1) Eugene L.Q. Lee: Temporal pattern processing is behaviorally and intergenerationally modulated by the tyraminergic/octopaminergic system in *C. elegans*
2) Montserrat Porta-de-la-Riva: Photon-based neuronal communication
3) Patrick Laurent: PPRP-1/PHACTR1 holophosphatase controls synaptic vesicle cycle in *C. elegans*
4) Mara Cowen: Multisensory integration and aggregation behavior depend on select synaptic adhesion molecules and glutamatergic signaling in a “hub-and-spoke” circuit

10:30 - 11:00: Coffee Break (catering on site)

Session Chair: Janet Richmond

11:00 - 11:30: Keynote lecture 6:
Meital Oren-Suissa
Design concepts of sexually-dimorphic circuits

11:30 - 12:30: Four Short talks from selected abstracts
1) Edgar Correa: The conserved transcription factor UNC-30/PITX is required to establish and maintain functional synapses in *C. elegans* GABAergic motor neurons
2) Elisa Frankel: Intracellular interactions recruit neurexin and drive presynaptic stabilization in *C. elegans*
3) Michal Ragan: Regulation of BK channel endocytosis by an RNF-145/EFA-6/ARF-6 molecular pathway
4) Kentaro Noma: Neuronal hyperactivation causes an age-dependent decline in associative learning behavior

12:30 - 13:30: Lunch (catering on site)

Plenary Session 5: Plasticity and Sensory responses
Session Chair: Karl Emanuel Busch

13:30 - 14:00: Keynote lecture 7
Piali Sengupta
The translatome of the AFD thermosensory neuron type links its activity history with neuronal and behavioral plasticity

14:00 - 15:00: Four short talks from selected abstracts
1) Sonu Peedikayil Kurien: Integration of neuronal activity and synaptic plasticity drives sexually dimorphic learning of pathogenic avoidance
2) Laura Molina-Garcia: PDF-1 modulation of aversion and reward during associative learning
3) WooKyu Kang: Nitric oxide produced by gut bacteria modulates foraging behavior through interaction with the oxygen sensing pathway
4) Ruhi Patel: Mechanosensory Behaviors of Skin-Penetrating Parasitic Nematodes

15:00-15:45: Women in science discussion

15:45 - 16:15: Coffee Break (catering on site)

Session Chair: Jihong Bai

16:15 - 16:45: Keynote lecture 8
Mario de Bono
Regulating the regulator

16:45- 17:45: four Short talks from selected abstracts

1) Canyon Calovich-Benne: Sensory neuron transcriptomes reveal complex neuron-specific function and regulation of *mec-2*/Stomatin splicing
2) Michael Iannacone: Viral infection in *C. elegans* promotes sleep, which is necessary for survival and energy maintenance
3) Mark Zhang: Insulin-like peptides control the dauer exit developmental decision in *C. elegans*
4) Benjamin Brissette: The *C. elegans* chemosensory system decodes complex blends of microbial metabolites to distinguish benign and pathogenic bacteria

19:00 - 24:00: Dinner excursion (Town Hall + party)

DAY 4, 27th July 2022

Plenary Session 6: Aging, Neural Diseases and Regeneration
Session Chair: Andrea Calixto

09:00 - 09:30: Keynote lecture 9
Yishi Jin
Understanding neuronal stress signaling

09:30 - 10:30: Four short talks from selected abstracts
1) Federica La Rocca: hnRNPQ/*hrpr-1* and RTN/*ret-1* role in splicing and neurodegeneration
2) Yoshiki Gabel Sakai: Histidine phosphorylation-mediated signal transduction regulates axon regeneration in *C. elegans*
3) Ho Christopher: Imaging age-related alterations in neuronal dynamics in *C. elegans*
4) Yan Xue: The metalloprotease ADM-4/ADAM17 promotes axonal repair

10:30 - 11:00: Coffee Break (catering on site)

Session Chair: Anne Hart

11:00 - 11:30: Keynote lecture 10
Maria Doitsidou
Using *C. elegans* to investigate the role of gut microbiota in Parkinson's

11:30 - 12:30: Four Short talks from selected abstracts
1) Wenyue Wang: An intestinal sphingolipid promotes neuronal health across generations
2) Evandro A de Souza: Anticipatory activation of the UPRER by pathogen-associated odour
3) Hamilton White: Impact of Traumatic Brain Injury on sensory neural function, behavior, and neural structure in *C. elegans*
4) Maria Belen Harreguy: Semaphorin signaling restricts neuronal regeneration

12:30 - 12:45: Closing remarks.

Sponsors

MEETING DESTINATION VIENNA
NOW ◆ TOGETHER

Vienna Convention Bureau

The Company of Biologists

https://www.biologists.com/

EMBO

European Molecular Biology Organization

ZEISS

ZEISS

SunyBiotech

SUNY BIOTECH

UNION BIOMETRICA

UNION BIOMETRICA

Keynote talks

Keynote talks are 25 minutes presentation plus 5 minutes questions.

Plenary Session 1: Technological Advances and Community Resources

Keynote lecture 1
Mei Zhen
Developmental strategies to maintain locomotory output
During development, animals can maintain behavioral output even as the underlying circuits structurally remodel. After hatching, C. elegans undergoes substantial motor neuron expansion and synapse re-wiring while the animal continuously moves with an undulatory pattern. We address how the locomotory circuit transitions from its juvenile to mature configuration without disrupting functional output through a combination of electron microscopy, optogenetics, and modeling. We outline multiple maturation strategies that are employed to support continuous maintenance of motor patterns for motor function robustness.

Plenary Session 2: Neurodevelopment

Keynote lecture 2
Shai Shaham
Astroglial control of *C. elegans* behavior
Abstract: Astroglia are implicated in the control of many behaviors, and their dysfunction accompanies pathological conditions manifesting behavioral abnormalities. Yet, neural circuit mechanisms by which astroglia affect behavior are largely unknown. The nematode *C. elegans,* with its simple nervous system and well-mapped connectome, is an attractive setting for deciphering such mechanisms. We have shown that *C. elegans* CEPsh glia, which associate with neuronal processes and synapses in the animal's brain (nerve ring), resemble mammalian astroglia in morphology, molecular biology, and function. We demonstrated extensive similarities between the development of the *C. elegans* nerve ring and formation of commissural tracts in the spinal cord, and identified conserved glial proteins driving nerve ring assembly, We also uncovered a key role for CEPsh glia in controlling a number of *C. elegans* behaviors, discovering that these astroglial cells perform a key function in blocking repetitive nervous system activation and associated behaviors. Loss of the glutamate transporter GLT-1, enriched in CEPsh glia and in mouse astrocytes, causes glutamate spillover, leading to ectopic presynaptic activation of the neuronal metabotropic glutamate receptor MGL-2/mGluR5. This, in turn, induces postsynaptic neuron oscillations, generating abnormal repetitive backing behavior. Importantly, murine GLT1 and mGluR5 are implicated in pathological motor repetition, suggesting that conserved mechanisms control repetitive behavior generation from *C. elegans* to mammals. Our studies establish *C. elegans* CEPsh glia as an exciting model to study fundamental molecular and cellular roles of astrocytic glia in neural circuit development and function.

Plenary Session 3: Neural Circuits and Behavior

Keynote lecture 3

Lidia Ripoll Sánchez, Jan Watteyne, Haosheng Sun, Robert Fernandez, Seth R Taylor, Alexis Weinreb, Mark Hammarlund, David M Miller III, Oliver Hobert, Isabel Beets, Petra E Vértes, **William R Schafer**

The neuropeptidergic connectome of *C. elegans*

Efforts are ongoing to build and map synaptic wiring diagrams, or connectomes, to understand the neural basis of brain function. However, chemical synapses represent only one type of functionally important signalling interaction between neurons; in particular, extrasynaptic neuromodulatory interactions involving neuropeptides are widespread in all nervous systems, and these wireless interactions are critical to the function of all animal brains. To probe the structure and function of these networks, we have generated a draft connectome of neuropeptide signalling in the C. elegans nervous system. By integrating single-cell anatomical and gene expression datasets with comprehensive biochemical analysis of receptor-ligand interactions, we have constructed a network representing ~32,000 signalling interactions between the 302 C. elegans neurons mediated by 91 neuropeptide-receptor couples. The resulting neuropeptide connectome is characterised by a high density of connections, extended signaling cascades, autocrine foci, and a decentralised topology. More than half its neurons participatw in an interconnected rich club of very high degree and three constituent communities sharing similar patterns of input connectivity. Intriguingly, although the main hubs of the synaptic connectome also have high neuropeptide degree, several of the most important nodes in this connectome are little-studied neurons that appear to be specialised for peptidergic neuromodulation. We anticipate that the C. elegans neuropeptidergic connectome will likewise serve as a prototype to understand basic organisational principles of neuroendocrine signaling networks in other animals, including humans.

Keynote lecture 4

Shingo Hiroki, Hirofumi Sato, Yu Toyoshima, Llian Mabardi, Hikari Yoshitane, Hinako Mitsui, Manami Kanamori, Chie Umatani, Shinji Kanda, Mashiro Tomoioka, Koichi Hashimoto, Hirofumi Kunitomo, Yoshitaka Fukada, Takeshi Ishihara & **Yuichi Iino**

Molecules and neural network underlying salt preference

C. elegans shows chemotaxis to NaCl, in which the direction of chemotaxis is determined by whether the salt concentration previously experienced in the presence of food was higher or lower than the current concentration in the environment. So how can worms generate opposing behaviors based on previous experience?

We've recently revealed the molecular overview of this switch; the presynaptic signalling pathway composed of diacylglycerol - protein kinase C (PKC-1) - syntaxin (UNC-64) acting in the sensory neuron ASER carries the short-term memory of salt concentration changes. This molecular signature affects the amount of glutamate released from ASER, which translates to the excitatory/inhibitory response of AIB, a postsynaptic neuron, through a sophisticated mechanism based on different sensitivity of excitatory and inhibitory glutamate receptors on AIB.

We also found that the response of AIY neurons, another class of ASE postsynaptic neurons, is driven by glutamate released from ASE, and they show experience-dependent response changes when stimulated by gradual changes of salt concentration.

Finally, we looked at overall dynamics of all neurons in the head, which revealed that salt stimulus information is spread via multiple pathways throughout many of the neurons in the head including those controlling motor programs and affects these dynamics in a probabilistic manner. These analyses move us forward toward a better understanding of sensory behaviors.

Plenary Session 4: Synaptic functions
Keynote lecture 5, The EMBO lecture
Jean-Louis Bessereau
The ins and outs of synaptic domain specification
At chemical synapses, spatial congruence between neurotransmitter release sites and post-synaptic receptor clusters is a key-parameter that shapes synaptic transfer function. Using a combination of pharmacological and visual genetic screens in *C. elegans*, we identified a series of synaptic proteins with unforeseen domain composition that control the identity of postsynaptic domains. Specifically, the extracellular matrix protein Ce-Punctin/MADD-4 is differentially secreted by cholinergic and GABAergic motoneurons and dictates the composition of postsynaptic domains. MADD-4 triggers the formation of extracellular scaffolding complexes. It also positions distinct transmembrane synaptic proteins that, in turn, recruit intracellular scaffolds to cluster acetylcholine and GABA receptors. I will provide an overview of this unanticipated molecular diversity and discuss it from an evolutionary perspective.

Keynote lecture 6
Meital Oren-Suissa
Design concepts of sexually-dimorphic circuits
The two sexes of a species can display marked differences in essential behaviors such as feeding or escape from dangers, but the mechanisms underlying such behavioral dimorphism are poorly understood. In our work, we trace the molecular and cellular events that generate sexually dimorphic circuits at the single synapse and gene level. For example, we find that in the circuit for nociceptive behaviors, rewiring of a single synaptic connection can determine the sexually-dimorphic behavioral outcome, while in the tail mechano-sensory circuit different cells and molecular mediators are employed in the two sexes to generate the same behavior. Our work suggests that conflicting evolutionary pressures introduced subtle changes into functional circuits to optimize the fitness of each sex.

Plenary Session 5: Plasticity and Sensory responses

Keynote lecture 7
Nathan Harris, Sam Bates, Jihye Yeon, Zihao Zhuang, Matt Bernstein, John Calarco, **Piali Sengupta**
The translatome of the AFD thermosensory neuron type links its activity history with neuronal and behavioral plasticity
Thermosensation is a critical sensory modality for all organisms. *C. elegans* robustly detects small temperature changes over a broad range and exhibits experience-dependent thermosensory behaviors. Thermosensation in the innocuous temperature range is mediated primarily by the single AFD sensory neuron pair whose temperature response and synaptic output thresholds are modulated as a function of the animal's temperature experience. I will

discuss our lab's ongoing work exploring the temperature experience-dependent transcriptional and post-transcriptional mechanisms that operate in this single sensory neuron pair to drive neuronal and behavioral plasticity.

Keynote lecture 8

Thanh Vuong-Brender, Sean M Flynn, Yvonne Vallis, S. Ece Sönmez, **Mario de Bono**

Regulating the regulator

The ubiquitous Ca2+ sensor calmodulin (CaM) regulates many proteins, including ion channels, CaM kinases, and calcineurin, according to Ca2+ levels. Although unappreciated, the combined amount of CaM binding proteins is much greater than that of CaM. This makes CaM availability an important variable in cells. How CaM levels are regulated is, however, unclear.

CAMTAs are CaM-binding transcription activators expressed broadly in nervous systems. Their loss confers pleiotropic behavioral defects in worms, flies, mice and humans. Disrupting *camt-1*, the sole *C. elegans* CAMTA, increases the excitability of sensory neurons; conversely overexpressing *camt-1* reduces stimulus-evoked responses in these neurons.

How CAMTAs regulate neurons is unclear. To address this question, we compared gene expression in several neuron types between *camt-1* mutants and wild-type. We identified hundreds of genes with altered expression in each neuron, but only two genes were consistently regulated by *camt-1* across neurons: CaM, and a long non-coding RNA. Most other changes in gene expression appear to be indirect consequences of altered neural activity.

We could rescue the behavioral and Ca2+ signaling defects of *camt-1* mutants by supplementing neuronal CaM levels using a heterologous promoter. Using ChIP Seq we showed that CAMT-1 binds three sites upstream of *cmd-1*, the *Ce* calmodulin; deleting these sites phenocopies *camt-1*.

Using CRISPR we created an operon at the *cmd-1* locus that co-expresses GFP11 from the same control elements that express CaM. Using pan-neuronally expressed GFP1-10 we showed that disrupting *camt-1* reduces CaM levels in most *Ce* neurons. We also find that CaM expression is regulated by neural activity, and by CaM levels in a negative feedback loop. Surprisingly, CAMT-1-GFP resides predominantly in the cytoplasm, suggesting its activity as a transcription factor is regulated.

We find that CAMTAs promote CaM expression not only in Ce but also in *Drosophila* and mouse. CaM levels in cerebellum are reduced in mouse mutants of CAMTA1. Our data suggest CAMTAs mediate a conserved and general mechanism controlling neuronal CaM levels, thereby regulating Ca2+ signaling, physiology and behavior.

Plenary Session 6: Aging, Neural Diseases and Regeneration

Keynote lecture 9

Yishi Jin

Understanding neuronal stress signaling

Neurons employ many mechanisms to sustain function throughout lifetime. Following traumatic axon injury, neurons can initiate timely and complex responses to regenerate and repair. Using laser axotomy assay, we have carried out large-scale genetic screening and discovered several molecular pathways induced by injury. Combined with live imaging, we characterized rapid cellular dynamics following axon injury. This talk will present our understanding of the genetic landscape responding to injury.

Keynote lecture 10

Maria Doitsidou

Using *C. elegans* to investigate the role of gut microbiota in Parkinson's

Parkinson's disease (PD) is characterised by the progressive loss of dopaminergic neurons in the substantia nigra, leading to the cardinal motor symptoms of the condition. Central to the pathology of PD is the accumulation of α-synuclein (α-syn) aggregates within Lewy bodies. In recent years, the human gut microbiota has immerged as an important factor influencing PD. Gut bacteria affect brain function by producing metabolites that enter the bloodstream, eliciting immune responses in the host or modulating neuronal function. A number of studies found notable differences in the microbiota of PD patients compared to healthy controls, which correlate with clinical features. Distinct gut microbiome signatures have also been observed in individuals with prodromal markers of PD. While microbiota was found to impact progression of the condition in murine models, the effects of single bacterial species remain poorly understood.

To tease apart contributions of individual bacterial species we use α-syn based *C. elegans* models of PD. We tested the effect of over 50 human gut bacterial species and probiotics for their effects on α-syn aggregation. We found that *B. subtilis* PXN21, isolated from a commercially available probiotic product, inhibits, delays, and reverses α-syn aggregation and associated toxicity. The probiotic diet also reduces α-syn induced dopaminergic neurodegeneration and behavioral defects.

We have identified several metabolic pathways differentially regulated in *C. elegans* fed with *B. subtilis,* of which alterations in sphingolipid metabolism contribute to the protective effect. Additionally, supplementing the regular *E. coli* OP50 diet with crude extract from *B. subtilis* cultures, leads to a significant reduction in α-syn aggregation in a dose-dependent manner, suggesting the involvement of one or more stable and extractable bacterial metabolite(s). We are using a combination of genetic and metabolomic approaches to identify these protective compounds. In parallel, we are assessing the efficacy of the probiotic in a mouse model of sporadic PD and in an ongoing double-blind placebo-controlled study for people with Parkinson's.

Short talks from selected abstract

Short talks are 12 minutes presentation plus 3 minutes questions.

Plenary Session 1: Technological Advances and Community Resources

Rapid and reversible optogenetic silencing of synaptic transmission by clustering of synaptic vesicles

Vettkötter, Dennis; Schneider, Martin; Liewald, Jana; Zeiler, Sandra; Guldan, Julia; Watanabe, Shigeki; Gottschalk, Alexander

The investigation of neuronal circuits, as well as molecular and cellular functions of neurons, requires the control of activity in a spatio-temporally precise manner. Optogenetics provides possibilites to stimulate or inhibit specific types of neurons with light. In past years, several tools were developed that allow inhibition of specific neurons on time scales from milliseconds to minutes or long-term silencing. However, these optogenetic tools come at the cost of slow induction or reversibility of neuronal function. Thus, a tool that allows for fast, long-term, and spatially restricted neuronal silencing, with fast reversibility, is of substantial need. Here, we designed an optogenetic tool to cluster synaptic vesicles (SVs) and thus inhibit their function acutely, by using the ability of the Arabidopsis thaliana cryptochrome 2 (CRY2) to form homo-oligomers. This novel tool, called optoSynC (optogenetic synaptic vesicle clustering), comprises CRY2, fused to the SV intrinsic membrane protein synaptogyrin (SNG-1). We benchmarked optoSynC at the behavioral and electrophysiological levels. Blue light illumination of pan-neuronally expressed optoSynC significantly reduced swimming cycles by 80% within 25 s. Recovery of swimming behavior could be observed after 15 minutes. Using a combination of optogenetic stimulation of neurons with the red-light activated channelrhodopsin Chrimson and blue-light induced inhibition using optoSynC, we show effective silencing even in a single neuron, PVD, required for nociception. Further, optoSynC can inhibit exocytosis for several hours, even at very low light intensities. Last, we employed electron microscopy to shed light on the mechanistic details of optoSynC. Analyzing the distances of SVs, we found that SVs moved closer together after optoSynC was activated by blue light, thus, confirming the clustering of SVs.
OptoSynC is a highly efficient, “non-ionic” tool for synaptic silencing, that might facilitate investigating SV clustering in the reserve pool, how they translocate to the plasma membrane, or in which precise sequence of events SV recycling proceeds.

Visualizing neuropeptide GPCR activation in C. elegans using PepSee

Watteyne, Jan; Cho, Sumin; Vandewyer, Elke; Geens, Ellen; Van Damme, Sara; De Fruyt, Nathan; Beets, Isabel

Neuropeptides are intercellular signaling molecules that function widely as neuromodulators [1]. Despite their importance in animal behavior and physiology, how they achieve their broad and often pleiotropic effects remains poorly understood. The C. elegans nervous system

encodes a dense neuropeptide signaling network, formed by more than 150 neuropeptide precursors and a similar number of neuropeptide GPCRs [2]. To further understand the functional organization of these pathways, we have adopted PepSee, a genetically-encoded sensor for GPCR activation, in C. elegans, which allows the spatiotemporal visualization of neuropeptide-receptor signaling. Specifically, neuropeptide GPCR activation is coupled to the production of a transcriptional nuclear-localized fluorescent reporter under blue-light illumination. The GPCR activation sensor shows stable fluorescent readout upon exogenous application of neuropeptide ligands, optogenetic stimulation of neuropeptide release, and under environments entailing endogenous neuropeptide signaling. Furthermore, GPCR activation can be visualized at both a multi-copy and single-copy sensor level, which can be modularly applied to different neuropeptide GPCRs. Using PepSee, we are investigating spatiotemporal signaling in the neuromedin U neuropeptide pathway. Using optogenetics, we found that conditional signaling of CAPA-1 neuropeptides, through activation of the neuromedin U receptor NMUR-1, underpins experience-dependent plasticity of salt chemotaxis behavior in C. elegans. CAPA-1 signaling from ASG neurons is specifically required for the retrieval, but not the acquisition, of learned salt avoidance [3]. In addition, distinct cells express either nmur-1 [4] or capa-1 depending on the animal's food context . Taken together, this highlights conditional and temporal aspects of neuropeptide signaling as important organizational motifs within the neuropeptide network, which we are further addressing with activity readouts of neuropeptide-receptor signaling. These findings and tools act as a scaffold to investigate how flexible behaviors and physiological responses emerge from neuromodulatory networks.

References

[1] van den Pol Neuron **2012**, (https://doi.org/10.1016/j.neuron.2012.09.014)

[2] Van Bael et al. J. Am. Soc. Mass Spectrom. **2018**, (https://doi.org/10.1007/s13361-017-1856-z) [3] Watteyne et al. Nat Commun. **2020**, (https://doi.org/10.1038/s41467-020-15964-9)

[4] Wibisono et al. Cell Rep. **2022** (https://doi.org/10.1016/j.celrep.2022.110321)

Acknowledgements

We thank the ERC for funding our research through project ERC-2020-STG 950328 and the KU Leuven for support.

Using genetic code expansion to develop a photo-activatable FLP recombinase.

Baxter, Kieran; Busack, Inka; Davis, Lloyd; Goutou, Angeliki; Tynan, Ailish; Bringmann, Henrik; Greiss, Sebastian

Sequence-specific DNA recombinases such as FLP and Cre are powerful and widely used tools for controlling gene expression. However, their application is currently limited by the availability of specific promoters to target their expression to cells of interest. We have recently developed a photo-activatable version of Cre which allows spatiotemporal control of gene expression with single-cell precision (Davis et al., 2021).

Here we present a method for generating and using a photo-activatable version of FLP recombinase using genetic code expansion. The photo- activatable FLP can be used as an alternative to, or in combination with, photo-activatable Cre, further expanding the set of tools for controlling gene expression in C. elegans.

Genetic code expansion refers to a method of incorporating non-canonical amino acids into proteins in vivo in a site-specific manner. We use photocaged amino acids, which contain a

'caging' group attached to the side-chain of an otherwise canonical amino acid. This caging group can render the protein containing the photocaged amino acid inactive, by blocking its active site. The caging group can be rapidly removed by illumination with 365nm light, which restores the canonical amino acid and allows for the photo-activation of the protein.
We have generated photocaged variants of FLP by replacing a critical residue within the active site with a photocaged amino acid, which blocks the activity of FLP. This FLP recombinase can be activated either globally, by illuminating the entire animal, or in individual cells using a 365nm laser. We use the photo-activatable FLP to drive expression of an optogenetic channel, allowing us to study the functions of the desired neurons.
Our system provides a valuable tool for the spatiotemporal control of gene expression in C. elegans, and can be used to study functions of individual neurons, or combinations of neurons, which otherwise could not be genetically targeted by other methods.
Davis, L. et al. (2021) 'Precise optical control of gene expression in C. elegans using improved genetic code expansion and cre recombinase', eLife, 10, pp. 1–22. doi: 10.7554/ELIFE.67075.

Automatically tracking feeding behavior in populations of foraging C. elegans
Bonnard, Elsa; Liu, Jun; Alvarez, Luis; Scholz, Monika; Zjacic, Nicolina

C. elegans feeds on bacteria and other small microorganisms which it ingests using its pharynx, a neuromuscular pump. Currently, measuring feeding behavior requires tracking a single animal, indirectly estimating food intake from population-level metrics, or using restrained animals. To enable large throughput feeding measurements of unrestrained, crawling worms on agarose plates at a single worm resolution, we developed an imaging protocol and a complementary image analysis tool called PharaGlow. We image up to 50 unrestrained crawling worms simultaneously and extract locomotion and feeding behaviors. We demonstrate the tool's robustness and high-throughput capabilities by measuring feeding in different use-case scenarios, such as through development, with genetic and chemical perturbations that result in faster and slower pumping, and in the presence or absence of food. Finally, we demonstrate that our tool is capable of long-term imaging by showing behavioral dynamics of mating animals over an hour. The low- resolution fluorescence microscopes required are readily available in*C. elegans* laboratories, and in combination with our python-based analysis workflow makes this methodology easily accessible. PharaGlow therefore enables the observation and analysis of the temporal dynamics of food intake and locomotory behaviors with high-throughput and precision in a user-friendly system.

Plenary Session 2: Neurodevelopment

Insulin-like signaling regulates left/right asymmetric synaptic connectivity
Tang, Leo Tsz-Ho; Lee, Garrett; Cook, Steve; Emmons, Scott; Bülow, Hannes

Lateral specialization of the central nervous system is a conserved and well-established feature across species, yet the underlying mechanism through which functional asymmetry arises remains largely unknown. Using a combination of EM reconstruction and fluorescent approaches, we have previously reported that the synaptic connection between the left/right pairs of salt-sensing neurons ASEs and odor sensing neurons AWCs in the nematode *C. elegans*

exhibit left-biased asymmetry, i.e. more synapses form from left ASE to AWC compared to right ASE to AWC neurons. We are using this paradigm as a model to explore the underlying mechanisms and functional implications of asymmetric connections. We found that this connection is plastic and responds to changes in salt concentrations. Adult animals exposed to salt concentration higher than that at larval development change the synapse number of the ASEàAWC connection in a concentration and time dependent manner, resulting in right-biased connectivity.
Furthermore, right-biased connectivity can also be induced by knockout of an insulin-like peptides (ILP), *ins-6/ILP* , indicating that synaptic connectivity is regulated by insulin signaling. An *INS-6* ::GFP reporter reveals that *ins-6* is expressed in two other pairs of neurons, ASIs and ASJs with left-biased asymmetric expressions in ASJ but not ASI neuron. Intriguingly, expression of the *INS-6* ::GFP in ASJ switches from a left bias to a right bias upon high salt exposure, demonstrating that *INS-6* ::GFP expression correlates with synapse number of the ASEàAWC connection. Using cell-specific knockout approaches, we found that *ins-6/ILP* expressed from ASJs, but not from ASIs, contributes to the asymmetry of the ASEàAWC connection. Furthermore, *ins-6* knockout in left ASJ only but not right ASJ induces the ASEàAWC connection to become right-biased. These results suggest that*ins-6/ILP* is acting in a paracrine manner to affect ASEàAWC connectivity. Using cell-specific knockouts approaches and reporters of insulin pathway activation, we also found that *ins-6/ILP* acts through the presynaptic ASE neurons to influence the ASEàAWC connection. In summary, our results shows that ASEàAWC synaptic connectivity responds to environmental changes in salt concentration that is mediated through changes in paracrine insulin signaling.

Syndecan, netrin, guidance receptors and Rho-family GTPases cooperate to regulate the number of neurites/cellular extensions in neurons and other polarized cells
DIMA, Raphael; BENARD, Claire; CHABI, Yann; Rivollet, Lise; Shaye, Daniel; Arena, Anthony; Bah Tahé, Marianne; Socovich, Alexandra

During development, neurons and other polarized cells establish precise morphologies that are critical to their function. Whereas mechanisms driving axon outgrowth and guidance are well understood, how neurite number is regulated in a developing neuron remains unknown. Heparan sulfate proteoglycans (HSPGs) consist of a protein core with attached heparan sulfate (HS) glycosaminoglycan chains which are known to regulate interactions between extracellular signals and their receptors to orchestrate cellular responses. Our analysis of mutants in the two C. elegans HS glycosyltransferases, rib-1 and rib-2, revealed the existence of a mechanism controlling neurite/cellular extension number through a striking defect that has been rarely observed. In these mutants, neurons that are unipolar in the wildtype can develop two neurites out of the soma, and the excretory canal cell can develop up to 8 canals instead of 4. Since axons and the excretory canals rely on common molecular mechanisms for the guidance of their migration, and because the supernumerary-canals defect is more penetrant, we have mainly studied the excretory canal cell as a first step towards elucidating the mechanism that controls neurite/cellular extension number. We find that the supernumerary cellular extensions form at the same time and have the same cytoskeletal organisation as normal canals in HSPG mutants. We have determined that the conserved HSPG Syndecan/SDN-1 is key in this mechanism, cell-autonomously controlling cellular extension number during embryogenesis. Our genetic analysis reveals that the guidance cue UNC-6 and the guidance receptors UNC-40, UNC-5 and SAX-3, all cooperate with SDN-1 to restrict the

number of cellular projections. Furthermore, we show that SDN-1 acts through two cytoskeleton modulators of the Rho-GTPases family CED-10/Rac and MIG-2/RhoG, which also function in the developing cell to control cellular extension number. Our findings uncover the existence of a HSPG-regulated system ensuring the establishment of proper cell extension number during polarized cells development. Given the evolutionary conservation of the developmental mechanisms implicated, this work provides information relevant to understanding the cellular and molecular bases of the development of precise cellular morphologies in varied cell types across animals, including neurons.

HDAC inhibition combats neurodevelopmental trafficking stressors
Taylor, Caitlin; Shen, Kang

Neurons are large, complex cells that face unique membrane trafficking challenges both during rapid developmental outgrowth and over the course of a long functional lifetime. We have previously shown that the recycling endosome protein RAB-10 and the unfolded protein response regulator IRE-1 are essential for dendritic development in the C. elegans sensory neuron PVD: loss of either rab-10 or ire-1 results in a dramatic reduction of the PVD dendritic arbor and defective trafficking of membrane proteins. The broad importance of membrane trafficking in neuronal health is underscored by many studies linking endolysosomal and secretory genes to both neurodevelopmental and neurodegenerative diseases, and we sought to uncover novel mutations that can protect developing neurons from membrane trafficking stressors. We performed a forward genetic modifier screen using the null allele of rab-10 and isolated a loss-of-function allele of sin-3 which rescued the dendritic defects of rab-10. sin-3 functions in a histone deacetylase complex (HDAC), which suggests a neuroprotective role for HDAC inhibition during dendritic development. HDACs are major regulators of chromatin structure that repress transcription by deacetylating histones, and we hypothesize that HDAC inhibition results in increased transcriptional flexibility that may be beneficial to combat cellular stress. We found that perturbing a SIN-3/HDA-3 complex rescued morphological and trafficking defects caused by both rab-10 and ire-1, suggesting that HDAC inhibition can compensate for multiple cellular stressors including ER stress and endolysosomal dysfunction. Our subsequent genetic and cell biological analyses suggest that HDAC inhibition protects against different cellular stressors via two distinct mechanisms, by altering endolysosomal trafficking outcomes and improving the processing of misfolded proteins. We hypothesize that SIN- 3/HDA-3 inhibition promotes alternative cell biological pathways, allowing diseased neurons to compensate for multiple membrane trafficking defects. We propose that transcriptional regulation of these compensatory developmental stress responses can help combat neuronal dysfunction.

Repurposing the Chromosome-Microtubule Coupling Machinery for Dendritic Branching.
Cheerambathur, Dhanya; Green, Mattie

Dendrite branching is an essential process for building complex nervous systems. A neuron's dendritic patterns govern the number, distribution, and integration of inputs. Though significant progress has been made in understanding the signalling pathways that pattern the dendrite, little is known about the intrinsic mechanisms involved in sculpting the branches. The microtubule cytoskeleton is critical to provide structure and exert force during dendrite

branching. Our study reveals an unexpected role for the kinetochore, the chromosome-microtubule machinery, in shaping the dendrites of the nociceptive receptor, PVD. The kinetochore is a highly conserved multiprotein complex whose canonical function is to connect chromosomes to microtubules during cell division. PVD dendritic morphogenesis is a dynamic process, and kinetochore proteins are localized along the dendrite and at branch points. Our studies show that kinetochore proteins play an essential role in establishing the dendritic pattern in PVD that is independent of its cell division function. The degradation of kinetochore proteins in PVD during early development results in dendritic fusion and overexpression of these proteins leads to loss of branching. The kinetochore protein associates with the endosomal marker rab-5, which is known to be a site of microtubule nucleation. Ectopic targeting of kinetochore proteins to the membrane causes filopodia-like structures to form. The findings suggest that kinetochore proteins play a role in the crosstalk between microtubules and actin-based structures that remodel dendrites. Presently, we are identifying neuron- specific protein interactors for the kinetochore proteins using proximity-based protein interaction methods. Taken together, these findings reveal an unexpected architect in dendritic branching and provide insight into how microtubules shape dendritic architecture.

The mind of a dauer: EM reconstruction for the dauer connectome
Yim, Hyunsoo; Choe, Daniel T.; Bae, J. Alexander; Nguyen, Ken C.; Kang, Hae Mook; Bahn, Sang-kyu; Hall, David H.; Kim, Jinseop S.; Lee, Junho

Dauer, an alternative stage of Caenorhabditis elegans, shows dramatic developmental plasticity. In harsh conditions, the worm chooses to survive first, instead of reproduction, and it is a significant trade-off for an individual to adapt to the environment. Since the dauer larvae show quite different physiological properties and behavioral patterns from non-dauers, it is strongly expected that the neural circuit of the dauer stage may have undergone neuronal remodeling. However, despite these specialties, the nervous system for the dauer has not been fully revealed until now. In this study, we acquired four dauer ssTEM(serial-section transmission electron microscopy) volumes. Image volumes were densely reconstructed to reveal the nervous system of a dauer, and lots of morphological changes were newly discovered in the dauer axons, such as branching, elongation, and contact changes that had not been previously reported. Deep-learning-based automatic synapse detection was developed to find synapses, and now an almost complete dauer connectome was revealed. Comparing the connectomes between non-dauer stages, we discovered differences in network properties and neuronal circuits. Especially, the connections of neurons involved in dauer entry and somatic-pharyngeal networking changed significantly. These findings show that massive rewiring happens in dauer, and they will contribute to the understanding of neuronal plasticity and adaptation to the environment of the organism.

Axonal mitochondria regulate gentle touch response through control of actin dynamics
Hegde, Sneha; Modi, Souvik; Awasthi, Anjali; Koushika, Sandhya

Mitochondria are present in the cell body, along neuronal processes and at the energy demanding regions of a neuron. Mitochondria are important for ATP production and buffering cytosolic calcium. Along the neuronal processes of *C. elegans* touch receptor neurons (TRNs),

we observe that about 80- 90% of axonal mitochondria are present at actin rich regions in L4 and young adults. The distribution of axonal mitochondria and F-actin change together across development. L1 animals lack uniform mitochondrial distribution which is achieved at L2 stage. This transition coincides with the changes in both the distribution of F-actin rich regions and its dynamics. We investigated the relationship between F-actin and axonal mitochondria. On constitutively depolymerizing F-actin with a genetic tool-deAct, the uniform mitochondrial distribution was lost. We used*ric-7* mutants that lack mitochondria in the axons to assess the relationship between mitochondria and actin. All*ric-7(lf)* alleles tested lacked F-actin dynamics along the axons. Artificially driving mitochondria along the neuronal process in *ric-7* mutants through a motor attached to mitochondria only in TRNs restored both uniform mitochondrial distribution and actin dynamics. The anti-apoptotic protein-CED-9 has been shown to regulate actin at synapses via mitochondria, we do not observe its role in axonal actin dynamics. To understand the importance of axonal mitochondria and F-actin, we checked for gentle touch response. L1 and deAct animals with a random mitochondrial distribution and no F-actin/F-actin dynamics respectively have decreased gentle touch responsiveness. Additionally, *ric-7(lf)* animals with no mitochondria along the neuronal process also showed defective gentle touch response. However, artificially driving mitochondria along the neurons restore the response to gentle touch stimuli suggesting that mitochondrially derived F-actin are important for gentle touch response. Expressing constitutively active form of RHO-1 in *ric-7(lf)* mutants in TRNs restored both actin dynamics and defects in gentle touch responsiveness. These data suggest that mitochondria along the neuronal process of TRNs are important for F- actin dynamics *in vivo* and mitochondria mediated F-actin dynamics is necessary and sufficient for response to gentle touch stimulation.

Temporal Maturation of the C. elegans Post-Embryonic Nervous System
Sun, HaoSheng

In most animals, the majority of the nervous system is generated and assembled into neuronal circuits during embryonic development. However, during juvenile stages, nervous systems still undergo extensive anatomical and functional changes to eventually form a fully mature nervous system by the adult stage. The molecular changes in post-mitotic neurons across post-embryonic development and the genetic programs that control these temporal transitions are not well understood. Using the model organism C. elegans, we comprehensively characterized the distinct functional states (locomotor behavior) and corresponding distinct molecular states (transcriptome) of the post-mitotic nervous system across temporal transitions from early post-embryonic periods to adulthood. We observed pervasive changes in gene expression, many of which are controlled by the developmental upregulation of the conserved heterochronic miRNA lin-4/mir-125 and the subsequent promotion of a mature neuronal transcriptional program through the repression of its target, the transcription factor lin-14. The functional relevance of these molecular transitions are exemplified by a temporally regulated target gene of the lin-14 transcription factor, nlp-45, a neuropeptide-encoding gene. We found that nlp-45 is required for temporal transitions in exploratory activity across larval stages, across sexual maturation, and into a diapause arrest stage. Combined, these studies provide new insights into regulatory strategies that control neuron-type specific gene batteries to modulate distinct behaviors states across temporal, sex and environmental dimensions of post-embryonic development, and also provide a rich atlas of post-embryonic molecular changes to uncover additional regulatory mechanisms.

ER network stability promotes organized microtubule disassembly during Compartmentalized Cell Elimination

Juanez, Karen; Jones, Madison; Sharmin, Rashna; Ghose, Piya

Programmed cell death is a critically important event for normal development and homeostasis. Morphologically complex cells are characterized by elaborate processes, such as axons and dendrites in neurons. While complex cells are very common, their programmed elimination is poorly understood, as is their elimination under pathological conditions or following injury. Microtubule (MT) disassembly is associated with region-specific elimination, also known as pruning, of morphologically complex neurons, but the exact nature of this relationship is unknown. We discovered a 'tripartite' killing program that eliminates the morphologically complex tail-spike cell (TSC) and the sex-specific CEM neurons during C. elegans embryonic development. This program, called Compartmentalized Cell Elimination (CCE), is characterized by three cell regions dying in three disparate ways. Of particular note, the single process/dendrite of these cells displays two very different elimination morphologies in its two segments. The proximal segment fragments in a manner strikingly reminiscent of developmental pruning or injury-induced Wallerian degeneration of axons; whereas the distal segment retracts, much like axons do following nutrient deprivation. Here we report that MTs have stereotyped dynamics throughout the development and death of the TSC. Through forward genetic screens, we found that genes promoting endoplasmic reticulum (ER) network stability, atnl-1/Atlastin and lnp-1/Lunapark, which encode the homologs of human Atlastin GTPase and Lunapark, promote process dismantling during CCE. We find that atnl-1/Atlastin and lnp-1/Lunapark promote the function of the conserved MT-severing ATPase SPAS-1/Spastin in facilitating CCE. Human Atlastin, Lunapark and Spastin are all associated with neurodegenerative conditions, including Hereditary Spastic Paraplegia. The MT reporter TBA-1 shows gross enrichment in the TSC process in ER network stability mutants compared to wild-type, suggesting hyperstability of MTs in these mutants. We propose that the stable ER network and ER network stability proteins anchor SPAS-1/Spastin to allow for precisely targeted and organized MT disassembly, which leads to the highly defined demise of the TSC process during CCE. Our findings shed new light on the localized elimination of complex cells and provide a mechanism for how MTs are linked to pruning and neurodegeneration through an unexpected connection with the ER.

Neuronal Mitochondrial Dynamics Coordinate Systemic Mitochondrial Morphology through Tyramine Signaling

Chang, En-Ni; Chen, Yen Ju; Pan, Chun Liang

Mitochondrial functionality among different somatic tissues is coordinated to achieve organismal physiological homeostasis and fitness. We recently found that perturbation of neuronal mitochondrial dynamics in neurons by disrupting fzo-1 Mitofusin, which is essential for mitochondrial fusion, triggered widespread mitochondrial fragmentation in the intestine and other peripheral tissues. This non-autonomous mitochondrial fragmentation requires serotonin, tyramine and autophagy/mitophagy gene activities, and it improves pathogen resistance under neuronal mitochondrial stress. Here we reported that specific receptors for serotonin and tyramine were involved in non-autonomous mitochondrial fragmentation.

Mutations in the tyra-3 tyramine receptor gene blocked non-autonomous mitochondrial fragmentation without affecting cell-autonomous mitochondrial fragmentation. Our data further showed that tyra-3 acted in the intestine, and is thus a receptor dedicated for neuron-gut communication of mitochondrial morphological control. Furthermore, mutations in hlh-30/TFEB and daf-16/FOXO significantly reduced non-autonomous mitochondrial fragmentation. Neuronal fzo-1 knockout (KO) triggered HLH-30 nuclear translocation in the intestinal cells, while the distribution of DAF-16 did not change under neuronal fzo-1 KO. HLH-30 acted both in the intestine and the neurons to regulate mitochondrial morphology in distal tissues. Our work identifies a neuron-gut signaling mechanism for coordinating mitochondrial morphology at the organismal level.(supported by the National Health Research Institutes, NHRI- EX111-11134NI; and the Ministry of Science and Technology, MOST 109-2320-B-002-019-MY3)

Early-life experience reorganizes neuromodulatory regulation of stage-specific behavioral patterns and individuality types during development
Stern, Shay; Ali Nasser, Reemy; Harel, Yuval

Transient early-life experiences may promote stereotyped behavioral effects across development, but also long-lasting behavioral responses that are variable among individuals, even when initially exposed to the same early-life stimulus. Here, we used longitudinal behavioral monitoring of C. elegans at high spatiotemporal resolution to study how starvation early in life shapes patterns of long-term behavior and individuality throughout all developmental stages. We revealed that early starvation generates distinct and discontinuous behavioral effects across different life stages that are mediated by segregated temporal regulation of dopamine and serotonin. While dopamine buffers long-term behavioral responses via specific receptors during intermediate developmental stages, serotonin promotes behavioral sensitivity during early and late stages. Interestingly, unsupervised analysis of temporal individuality patterns across development uncovered multiple temporal individuality types that coexist within the isogenic population and further identified neuromodulatory effects on their composition following stress. These results show that early-life experiences expose stage-specific behavioral plasticity and altered individuality structures across developmental timescales.

Plenary Session 3: Neural Circuits and Behavior

Mapping the Functional Connectome in C. elegans
Dvali, Sophie; Randi, Francesco; Sharma, Anuj; Leifer, Andrew;

Measuring how signals propagate through the nervous system is important for understanding how neural dynamics are generated and uncovering how neuroanatomy relates to neural function. Here we sequentially perturb individual neurons in C. elegans optogenetically and simultaneously measure the response of all neurons in the brain via calcium imaging in order to measure functional connectivity at whole-brain scale and cellular resolution. We measure the sign, strength, causal relations, and temporal properties of effective connections between neurons. We present a preliminary functional connectivity map covering 90 of 118 neuron

classes recorded from 45 animals. The functional map recovers properties of previously well-studied circuits and connections, such as AFD to AIY, and provides clarity to other connections that had remained ambiguous.
Our measured functional description of the network better predicts correlations in spontaneous neural activity than an anatomical one. To investigate differences between the anatomical and functional descriptions, we constructed detailed simulations of whole-brain neural dynamics that are constrained either by anatomy or by our measured functional connections and found that the two make different predictions of neural dynamics.
Neural connections that are extra-synaptic and therefore not visible to anatomy may explain some of the differences we observe between the anatomical and functional descriptions of the network. We investigated responses to activation of neuron RID, which has very few outgoing anatomical connections, but which is thought to communicate via neuropeptides released extra-synaptically from dense-core vesicles. We measured functional connections between RID and other neurons that express receptors for peptides released by RID. We found that the responses of these neurons to stimulation of RID were reduced in unc-31 mutants, that are deficient for dense-core vesicle mediated extra-synaptic signaling, when compared to the wildtype. This provides direct evidence that RID communicates extrasynaptically and, importantly, these connections are captured by our functional connectivity map.

A circuit for head-body coordination during forward locomotion
Ahamed, Tosif; Hung, Wesley; Chang, Maggie; Wang, Ying; Mulcahy, Ben; Meng, Jun; Woo, Shifei; Sathaseevan, Anson; Samuel, Aravinthan; Zhen, Mei; Lu, Yangning

Head and body movement in *C. elegans* is controlled by distinct motor circuits that can operate independently of each other. Coordinated activity between these two circuits is essential for locomotion. However, how these circuits couple and uncouple to coordinate motion patterns along the worm's body remains largely unknown. First, to address the challenge of studying behavioral differences at different body segments along the body, we developed a new analysis pipeline for a complete spatiotemporal characterization of*C. elegans* body wave dynamics. With this pipeline, we show that a subcircuit composed of gap-junction connected interneurons AVG and RIF coordinate the head and body during forward locomotion. We show that stimulation of this subcircuit leads to faster locomotion and increased activity in head motor-neurons and AVB premotor interneurons. Consistent with their role in head-body coordination, AVG stimulation in AVB ablated animals silences the body but leads to increased head bending. While AVG stimulation with simultaneous silencing of head muscles leads to body undulation without head movement. As an ethological consequence of head- body coordination, we investigated foraging behavior, which is composed of two motor states roaming and dwelling. Coordinated head-body undulation during roaming state facilitates efficient forward movement, resulting in wider exploration of the food-plates. Whereas dwelling, a low centroid-displacement state, is characterized by uncoupling of the head and body. Further demonstrating their role in head-body coordination, we find that genetic perturbations to the AVG/RIF circuit made the animals more likely to dwell, compromising their ability to efficiently explore food plates by roaming. In summary, our work reveals an important behavioral role for the previously uncharacterized neuron AVG, while simultaneously revealing a neural circuit for the coordination of head and body motor patterns in *C. elegans.*

FLP-17 neuropeptide and its cognate receptor EGL-6 regulate a novel C. elegans oviposition behavior that increases reproductive fitness

Lee, Tongyoung; Lee, Jin; Yoon, Kyoung-hye

Parental behaviors are an evolutionary adaptation for the benefit of offspring survival. Here, we identified a novel maternal oviposition behavior when cultivated in a 3D environment that increases the survival of the young. C. elegans lays eggs directly on the OP50 lawn with no discernable pattern in 2D NGM conditions. In 3D conditions, however, the mothers display a stereotypical behavior remaining near the edge of the spherical bacterial colony, temporarily leaving the area to lay her eggs far away from the bacteria. This results in a ring of eggs located almost 1 mm outside the bacteria.
Performing a candidate mutant screen, we show mutants of flp-17, which encodes a FMRF-like neuropeptide expressed solely in the BAG sensory neurons, and egl-6, which encodes the cognate receptor of FLP-17 in the HSN motor neurons, were defective for oviposition behavior, laying eggs close to the bacteria. Transgenic rescue of flp-17 restores normal oviposition behavior. In addition, genetic ablation of the BAG and HSN neurons in egl-46 and egl-1 mutants, respectively, inhibits oviposition behavior. Previous studies have shown that FLP-17 is involved in both O2 and CO2 dependent sensation, and we demonstrate that due to the embedded bacteria, the embedded worms are likely exposed to a low oxygen environment in 3D. We tested egg-laying in mother worms in various gas conditions in a 2D environment and determined that 7% O2 could induce an oviposition behavior in a flp-17-dependent manner. We wondered whether oviposition behavior somehow increased the reproductive fitness of the mother in a 3D environment and showed that flp-17 mutants had significantly decreased brood size in 3D compared to 2D NGM plates. Finally, we placed eggs inside and outside a concentrated OP50 lawn in low oxygen conditions and evaluated survival of the young. We demonstrated that eggs placed inside the lawn mostly died, whereas most of the eggs placed outside survived. Overall, we describe an oxygen-sensing circuitry mediated by a neuropeptide and its cognate receptor that alters egg-laying behavior to increase survival of the young in toxic environments.

Closed-loop interrogation of whole-brain dynamics for causal analysis of neural network activity and behavior

Dunn, Raymond; Miller, Julia M.; Borchardt, Jackson; Bubnis, Gregory; L'Etoile, Noelle; Kato, Saul

The C. elegans brain is a nonlinear control system, which combines time-varying sensory input from the environment with evolving internal state to produce effective continuous behavioral output. Whole-brain calcium imaging has revealed that the neuronal network cycles through broadly distributed patterns of activity which correspond to major motor behavioral states, such as forward and reverse crawling and turning. However, whole- brain dynamics studies have been largely correlative, motivating our desire to study the effect of perturbation on global state evolution in order to determine the causal aspects of neural network activity.
We ask whether behavioral state sequences are determined by the time evolution of low-dimensional network state; we term this the stable manifold hypothesis, which predicts that certain perturbations of network state should, at specific times relative to the global brain state cycle, influence the future behavioral state of the animal. We describe the design and

implementation of our closed-loop system to address these questions, via measurement and analysis of whole-brain neural activity in real-time to determine the timing of direct optogenetic activation of single neurons, combining the approaches of genetically restricted expression of a red-shifted depolarizing opsin and patterned illumination with a digital micro-mirror device (DMD). We demonstrate the successful optogenetic activation of several neurons including OLQV, OLQD, SMDV, SMDD, ASH, ASI, RIA, and RIV while simultaneously recording whole-brain activity. We track depolarization of the AVA neuron as a fictive readout of reversal. We found that transient activation of the sensorimotor neurons SMDV or SMDD immediately halts an ongoing reversal in roughly 30% of trials, despite lacking direct connectivity to AVA. Building on this finding, we applied machine learning methods to characterize the dependence of influenceability on the evolving network state to test the stable manifold hypothesis of action selection. By extending this perturbative approach to more neurons and brain cycle times, we can map out regions of causal influenceability in neural state space and thereby uncover causal mechanisms of neurobehavioral control.

Internal state modulates brain-wide representations of behavior in C. elegans
Kim, Jungsoo; Atanas, Adam

The brain constantly fluctuates among a wide range of internal states that modulate how sensory cues are processed to give rise to behavior. Recent studies have shown these states are broadly reflected in neural activity across many brain regions. In addition, moment-by-moment behavioral variables are also represented in neural activity across many brain regions. This gives rise to a view that neural representations of internal states and acute behavioral variables co-exist in most brain regions, but how internal states impact the neural encoding of behavior remains largely unclear.
In this study, we address this question using the nematode C. elegans, whose transparent body and well-defined neural circuits allow us to analyze brain-wide representations of behavior across a range of internal states. First, we engineered a microscope that can capture whole-brain activity along with comprehensive behavioral information of a freely-moving animal. Using custom software, we can extract calcium signals from these datasets with extremely high signal-to-noise in a fully automated fashion. These neural datasets, combined with comprehensive behavioral quantification (locomotion, feeding, posture, etc), allow us to map the intricate relationship between brain-wide neural activity and behavior over varying internal states. To determine how each neuron encodes specific behavioral features, we developed a flexible nonlinear encoding model that can fit almost all of the neurons carrying overt behavioral information. Analysis of the resulting models reveals that neurons can encode the worm's behavior over a wide range of timescales, encode multiple behaviors simultaneously in a nonlinear yet stereotyped fashion, and modulate their encoding of behavior depending on the animal's internal state. Our results provide a global view of how circuits across the brain encode each motor parameter of an animal, and how changing internal states flexibly modulate these encodings.

Neuropeptide NLP-47 and its receptor GNRR-1 promote forgetting of olfactory memory in C. elegans
Onishi, Yuuki; Teo, Jamine; Kitazono, Tomohiro; Ishihara, Takeshi

Animals acquire and store information as memories that are required for their behavior and decision-making. To mitigate undesirable effects of old information stored in their brain, they must forget some dispensable memories. However, molecular mechanisms in forgetting are still unclear. To investigate the mechanisms of forgetting, we use olfactory learning in C. elegans as a model. C. elegans is highly attracted to some odorants such as diacetyl, although, after prolonged exposure to odorants without food, the animals adapt to the odorants and show weak chemoattraction. The adapted animals can regain their chemoattraction after the cultivation on food for several hours, and this recovery can be considered as forgetting. Previously, our studies showed that TIR-1/JNK-1 pathway in AWC sensory neurons accelerates forgetting of olfactory memory through releasing of "forgetting signals". However, the molecular basis of "forgetting signals" remains elusive.
Our previous study implies that neuropeptides might be responsible for forgetting signals. Therefore, to identify neuropeptides that serve as "forgetting signals" from AWC neurons, we searched for genes by using CeNGEN (C. elegans Neuronal Gene Expression Network, a dataset of single-cell RNA sequencing), and found 15 candidate of neuropeptide genes which are expressed significantly higher in AWC. By using CRISPR-Cas9, we created these mutants and analyzed their forgetting phenotype. Among these candidates, neuropeptide nlp-47 mutants showed forgetting defect, although they show normal chemoattraction and adaptation to diacetyl as wild-type. Moreover, injection of wild-type nlp-47 genomic fragments could recover forgetting phenotype in the mutants. These suggest that neuropeptide NLP-47 is responsible for accelerating forgetting. NLP-47 is one of the neuropeptide-like protein and it is known to be received by GNRR-1, an orthologue of human GnRH receptor. In fact, in mammals, GnRH receptor is expressed in rat hippocampus, which is an important area for memory, however, function of GnRH receptor in memory is elusive across species. Thus, I checked forgetting phenotype of gnrr-1 mutants and found that they showed the forgetting defect. It suggests that GNRR-1 may also be involved in promoting forgetting. Further analyses of these factors will reveal how memory forgetting are regulated by forgetting-promoting signals in animals.

How worms explore 3D space

Cohen, Netta; Ilett, Tom; Yuval, Omer; Salfelder, Felix; Holbrook, Robert; Ranner, Thomas; Hogg, David

From microorganisms to animals, navigation and exploration of the natural environment requires a variety of locomotion gaits that are combined and modulated across a wide range of time scales. *Caenorhabditis elegans* lives in granular and complex fluid habitats which it must explore and forage for survival. However, the nature and mechanisms of its explorations are largely unknown in volumetric environments. In studies of planar motion of*C. elegans* , local area search is well described in terms of tumble and run dynamics consisting of undulations (runs) separated by random turning events. In 3D neither the locomotion primitives nor the exploration strategies are known. Here we present a high resolution triaxial recording pipeline for capturing both microscopic postures and macroscopic trajectories across a range of homogeneous non-Newtonian environments. Using our new corpus of reconstructed postures and trajectories, we identify non-planar undulatory behaviours, as well as non-planar manoeuvres, including new reversals and new turning behaviours. We find that *C. elegans* explores its local volume using a nested hierarchy of these locomotion gaits and manoeuvres.

Finally, we show that this volumetric exploration can be explained with a simple three-state model that uses rates which exhibit a strong separation of timescales. These results demonstrate that hierarchies of timescales and non-planarity are essential components of foraging and survival in the worm's natural environment.

Mechanosensory feedback regulates egg-laying circuit activity and behavior of C. elegans
Medrano, Emmanuel; Collins, Kevin

Egg laying in C. elegans is a two-state behavior regulated by sensory input. Unfavorable environmental conditions and nociceptive mechanosensory neurons inhibit egg-laying circuit Ca2+ activity and promote the inactive behavior state. How the egg-laying circuit becomes activated is less clear. Our recent results show that changes in egg accumulation in the uterus alter burst-firing activity of the serotonergic HSN command neurons, suggesting a stretch-dependent homeostat promotes bouts of egg-laying. Here, we test the hypothesis that mechanosensory feedback of egg accumulation promotes the active behavior state. Using an acute microinjection technique to mimic changes in pressure and stretch caused by egg accumulation, we find that injection rapidly stimulates vulval muscle contractility and egg release. Microinjection induces Ca2+ transient activity in all cells in the egg- laying circuit, but we find that vulval muscle Ca2+ activity does not require synaptic transmission from the HSN or VCs and is still observed in unc-13 or unc-31 mutants lacking neurotransmitter or neuropeptide release, respectively. Vulval muscle Ca2+ responses are reduced in egl-19 mutant animals or in animals treated with nemadipine, an L-type channel-specific blocker. Direct prodding of the vulval muscles similarly triggers Ca2+ responses, suggesting the vulval muscles themselves are the direct targets of the stretch-dependent mechanical stimulus. Together, we propose a model for a stretch-dependent homeostat driven by feedback of egg accumulation in the uterus that regulates vulval muscle Ca2+ activity for egg laying. A retrograde signal from the activated vulval muscles then sustains HSN activity and the active state for as long as there are eggs to lay.

Overactivation of a sleep-active neuron decouples survival from the need to sleep
Busack, Inka, Henrik Bringmann

Sleep is a state of behavioral quiescence that is closely associated with survival. Sleep-active neurons promote sleep and survival. It is not known, however, whether sleep-active neurons need to cause sleep to promote survival. Here, we tested how depolarization of the sleep-active RIS neuron in Caenorhabditis elegans controls sleep and survival during larval starvation. Survival always increased with raised RIS depolarization. RIS depolarization promoted sleep over a long range. Unexpectedly, however, high levels of RIS depolarization caused a nearly complete loss of sleep. Similarly, overexpression of sleep-inducing FLP-11 neuropeptides in RIS inhibited sleep, indicating that overactive transmission from RIS inhibits sleep.
Despite the loss of sleep, survival was normal following FLP-11 overexpression. Thus, RIS overactivation abolishes sleep yet supports survival. These results indicate that regulation of sleep and survival are separable functions of a sleep-active neuron that are normally coupled but can be uncoupled by sleep-neuron over activation.

Encoding principles of a compact sensory system
Bokman, Eduard; Pritz, Christian; Ruach, Rotem; Itskov, Eyal; Zaslaver, Alon

Animals critically depend on accurate sensation and processing of environmental cues. This task becomes particularly challenging for animals with a compact and highly interconnected sensory system. Here, we used C. elegans to investigate how sensory information is encoded within a small nervous system. For this, we generated a strain expressing the genetically encoded calcium indicator GCaMP in all 60 ciliated neurons, and used a fast-scanning confocal system to measure activity simultaneously from all chemosensory neurons while subjecting the worms to various stimuli. We found that the sensory system responds with small, unique, and near-perfectly bi-laterally symmetrical subsets of neurons. Analysis of mutants, defective in neuro- transmitter or neuro-peptide release, revealed that the number of primary neurons which directly respond to individual cues is minimal, typically consisting of 2-3 neuron types. Moreover, sensory neurons exhibit a range of response dynamics that are both stimulus- and circuitry-dependent, effectively increasing encoding capacity of the compact sensory system. Finally, exposing animals to odor mixtures revealed that individual sensory neurons employ a variety of complex integration strategies, including summation, averaging, and even a NOT(AND) logic-gate-like computation. Taken together, our results elucidate the principles that allow a small and compact sensory system to expand its encoding repertoire and to efficiently extract information from the environment.

Plenary Session 4: Synaptic functions

Temporal pattern processing is behaviorally and intergenerationally modulated by the tyraminergic/octopaminergic system in C. elegans
Lee, Eugene L.Q.; Horvitz, H. Robert

The ability of an animal to recognize patterns in the timing of stimuli in its environment, associate the relevant events, and respond appropriately when subsequent events reoccur is key to survival. How molecular and cellular factors interact to define relevant timescales and react in proper sensory “time windows” is a fundamental problem. I have designed a novel trial-by-trial associative learning paradigm that allows responses to timing patterns to be investigated using *C. elegans* . *C. elegans* can associate a neutral odor stimulus with a noxious light stimulus when they are paired in time, and worms respond differentially when the ordering of these two stimuli is varied. Different temporal structures of exposures can be generated by introducing timing gaps between the odor and the light. Conditioning that occurs when the neutral odor is presented some time after the light is known as trace-conditioning, as opposed to standard Pavlovian conditioning that occurs when there is no temporal gap. Worms can perform trace- conditioning and are decreasingly sensitive to increased trace periods. Human trace-conditioning is thought to be associated with “awareness,” as opposed to standard-conditioning, which does not require the subject to be aware of the predictive relationships between stimuli. From a candidate screen of biogenic amine neuromodulators, I found that dopamine, serotonin and tyramine/octopamine are all involved in controlling the standard- conditioning response. However, only the tyraminergic/octopaminergic system modulates trace-conditioning. Adapting to the environment is crucial not only for the survival of an individual but also critical for its future progeny. Worms exposed to learning in the

parental generation produced progeny with altered trace responses. The order and timing of the stimuli pattern encountered dictated this intergenerational adaptation: only worms exposed to ordered odor-light stimuli modulated intergenerational timing adaptation. Worms experiencing identical levels of odor-light stimuli but in a randomly shuffled pattern showed no intergenerational adaptation. Intriguingly, tyramine seems to be involved in this intergenerational inheritance. Determining the mechanisms that enable a purely cognitive element such as patterned timing recognition to affect intergenerational modulation of responses to timing patterns might uncover new biology that informs us about how parental experiences can impact future generations.

Photon-based neuronal communication

Krieg, Michael; Porta-de-la-Riva, Montserrat; González, Adriana Carolina; Sanfeliu-Cerdán, Neus; Karimi, Shadi; Morales-Curiel, Luis Felipe; Malaiwong, Nawaphat; González-Bolívar, Sara; Fernández, Pablo; Hurth, Cedric

Cell communication is basic for the development of multicellular living organisms as well as for their correct homeostasis. Miscommunication is the cause of many diseases and can even lead to cell death. Neural cells are not an exception and failure in neuronal signal transmission leads to diseases of different nature, from depression to schizophrenia or Parkinson. In this work, we describe a powerful and versatile cell communication system based on light, which we have named PhAST for Photon-Assisted Synaptic Transmission. PhAST relies on the expression of a calcium sensitive luciferase that emits light upon intracellular increase of calcium and excites a linked fluorescent protein that, not only tunes the color, but also greatly amplifies its output light. The coupling of this NanoLantern (NL) to a postsynaptic light sensitive ion channel allows depolarization and signal transmission to the downstream neuron. We have successfully expressed this system in the nose touch pathway of *C. elegans* , overcoming a cell specific glutamate defect in ASH, the main nose touch effector, using a blue NL coupled to extremely sensitive CHR2 variant. In addition, the expression of PhAST in this sexually dimorphic pathway increases the nose touch sensitivity of wild type males almost to the levels of hermaphrodites. Lastly, coupling a green NL to the ACR1 anion channelrhodopsin in this same circuit caused a change in polarity, overwriting the endogenous reversal signal and completely abolishing the response to nose touch.

PPRP-1/PHACTR1 holophosphatase controls synaptic vesicle cycle in C. elegans.

Laurent, Patrick

The activity-dependent fusion, retrieval, and recycling of synaptic vesicles (SV) is essential for neurotransmission. A forward genetic screen to identify genes involved in neuromodulation by neuropeptides isolated snn-1/synapsin and pprp-1/PHACTRs. Synapsins are key phosphoproteins involved in SV recycling and SV clustering, Synapsins mutations are associated to seizure in mouse and human. PHACTRs form holophosphatases together with Protein Phosphatase 1 (PP1) when G-actin get depleted. PHACTR1 autosomal dominant variants are associated to Developmental Epilepsy and Encephalopathy 70 (DEE70). Using C. elegans, we dissect pprp-1/PHACTRs structure and neuronal functions in-vivo. Mimicking DEE70 mutations in pprp-1 gene generate constitutively active holophosphatase by reducing its inhibition by G-actin. The PPRP-1-PP1 holophosphatase inhibits neurotransmission at

neuromuscular junction by a mechanism that include the control of Synapsin phosphorylation: Serine 9-Synapsin is dephosphorylated in pprp-1(DEE70) while it is hyperphosphorylated and spread in the axon in pprp-1(null). pprp-1(DEE70) modify the Synaptic Vesicle (SV) cycle: reuptake of SV membrane protein by local endocytosis is reduced, faster synaptic fatigue was observed that likely corresponds to exhaustion of SV recycling as we observed a reduced number of SV at synapses and abnormal endocytic patterns by EM of the NMJ. In our model, G-actin depletion occurring within synaptic bouton is used as a signal for holophosphatase formation and dephosphorylation of substrates important for SV recycling or clustering. DEE70 variants would constitutively activate this presynaptic mechanism.

Multisensory integration and aggregation behavior depend on select synaptic adhesion molecules and glutamatergic signaling in a "hub-and-spoke" circuit
Cowen, Mara; Hart, Michael; Chalasani, Sreekanth; Reddy, Kirthi

How synaptic adhesion molecules regulate circuit dynamics and contribute to multisensory integration remains elusive despite its clear evolutionary importance. As the connectome in C. elegans is mapped and the neural circuits controlling many behaviors are known, we asked how mutations in synaptic adhesion genes alter downstream behavior of a well-studied sensory integration circuit. The RMG "hub-and-spoke" circuit consists of several sensory neurons synapsing onto the RMG interneuron through electrical and chemical connections. Worms lacking npr-1 display aggregation, which depends on the integration of aversive stimuli, oxygen, pheromones, CO2, and other sensory cues by sensory neurons and RMG. We find that the singular C. elegans ortholog of the synaptic adhesion gene, neurexin (nrx-1/NRXN1), is required for aggregation in npr-1 mutants. Disruption of nrx-1 (null and a-isoform specific mutant alleles) in npr-1 animals leads to a significant reduction in aggregation behavior. Re-expression of the a-isoform of NRX-1 in only two pairs of sensory neurons within the RMG circuit rescues aggregation behavior of nrx-1 null mutants. These sensory neurons signal noxious stimuli via glutamate to RMG and other interneurons. Loss of glutamate signaling (eat-4 null) in npr-1 animals reduces aggregation, implicating glutamate in this behavior for the first time. We find that loss of nrx-1 and eat-4 leads to a decrease in the number of cla-1 synaptic puncta in the aversive sensory neuron(s) relative to npr-1 controls. Further, we find that disruption of the single neuroligin (nlg-1) in C. elegans (the canonical binding partner of neurexin and a second synaptic adhesion gene) decreases aggregation in npr-1 mutants; and nrx-1; nlg-1 double mutants show an additive reduction of aggregation behavior. Interestingly, disruption of a third synaptic gene, shank (shn-1/SHANK), does not alter aggregation, suggesting unique functions for synaptic molecules in regulating multisensory integration. This work defines novel roles for synaptic genes in regulating proper signaling in sensory integration circuits, identifies a novel role for glutamate signaling in aggregation behavior, and helps establish a mechanism for altered behavior in nrx-1 and nlg-1 mutants. This behavioral paradigm may also serve as a future platform to test the functional impact of specific human mutations.

The conserved transcription factor UNC-30/PITX is required to establish and maintain functional synapses in C. elegans GABAergic motor neurons.
Correa, Edgar; Mialon, Morgane; Pinan-Lucarre, Berangere; Bessereau, Jean-Louis; Kratsios, Paschalis

Neuronal communication critically depends on the establishment and maintenance of functional synapses. This process relies on the ability of presynaptic neurons to synthesize, package, and release a neurotransmitter, and the ability of postsynaptic neurons to present the correct
neurotransmitter receptors. Nevertheless, the molecular mechanisms that coordinate these distinct events, occurring at a pre- and post-synaptic cell, are poorly understood. C. elegans represents an ideal model to address this knowledge gap, due to its known connectome, powerful genetics, and single-cell resolution analysis. The evolutionarily conserved transcription factor (TF) UNC-30/PITX1-3 has been shown to control neuronal communication between nerve cord GABAergic (GABA) motor neurons (MNs) and body-wall muscle by directly activating the expression of GABA biosynthesis genes (e.g., unc-25/GAD, unc-47/VGAT) in the presynaptic side. Our preliminary data indicates that UNC-30 is also required for proper GABA-Receptor (GABAR) clustering on the postsynaptic side. Intriguingly, the same GABAR clustering defect is observed in animals lacking the short isoform of MADD-4/Punctin (madd-4S), a secreted synaptic organizer produced by GABA MNs. We hypothesized that UNC-30 controls the establishment of functional synapses by activating madd-4S. Indeed, madd-4S expression is reduced in GABA MNs of unc-30 mutant animals. ChIP-Seq data suggests UNC-30 controls madd-4S directly, a notion we confirmed by deleting a region containing an UNC-30 binding site in the context of the endogenous madd-4S locus. Because unc-30 and madd-4S are continuously expressed in GABA MNs, we next asked whether UNC-30 is required to maintain expression of madd-4S. Through temporally controlled UNC-30 protein depletion, we found this to be the case, suggesting UNC-30 is continuously required to maintain GABAR clustering. Besides acting as a transcriptional activator, our preliminary data suggests that UNC-30 prevents the adoption of alternative neuronal identities in GABA MNs. We found that several genes (e.g., madd-4L/Punctin, glr-5/GluR, unc-53/Nav2), normally expressed in cholinergic MNs, become ectopically expressed in GABA MNs of unc-30 mutant animals. Altogether, these findings uncover a dual role for UNC-30: acting as direct activator of madd-4S and GABA biosynthesis genes, and repressor of alternative identities in GABA MNs.

Intracellular interactions recruit neurexin and drive presynaptic stabilization in C. elegans
Frankel, Elisa; Kurshan, Peri; Tiroumalechetty, Araven; Sequelle Henry, Parise; Su, Zhaoqian; Wu, Yinghao

Neurexins are synaptic cell-adhesion molecules (sCAMs) highly associated with autism and schizophrenia. sCAMs have long been thought to mediate the formation of neuronal circuits by recognizing trans-synaptic partners and initiating the assembly of synaptic specializations. However, our group and others have found that the loss of sCAMs does not lead to the elimination of synapses, but instead to defects in synapse stability, maturation, and plasticity. We now report that a short and conserved isoform of neurexin lacking any canonical trans-synaptic binding domains is the dominant isoform of neurexin expressed in C. elegans, and that neurexin's intracellular domain alone is sufficient to mediate its presynaptic maturation and stabilization functions. Moreover, we find that endogenously-tagged neurexin is clustered at synapses through intracellular interactions, and identify the conserved PDZ-containing scaffold SYD-1 as critical for neurexin clustering. How then, might cytosolic scaffolds themselves become associated with the membrane prior to neurexin arrival? Using computational modeling, we predict that SYD-1 can interact directly with the membrane via

its C2 domains. Together, these data suggest a model in which scaffold molecules rather than sCAMs are the initiators of presynaptic assembly, and that subsequent binding of neurexin via its PDZ-binding motif serves to stabilize this interaction at the membrane. In support of this model, we find that SYD-1's arrival at nascent synapses precedes the arrival of neurexin. Finally, we demonstrate that the long and short isoforms of neurexin are differentially expressed throughout development (with wider expression of the short isoform at earlier stages), suggesting that they play specific roles in synapse formation and specificity. Together these results provide a mechanism by which synapse assembly can be initiated at the plasma membrane before sCAM arrival. Moreover, we identify a hitherto unappreciated contribution of neurexin's intracellular domain to its synaptic localization as well as to its function in presynaptic stabilization, with implications for understanding neurexin-associated neurodevelopmental disorders.

Regulation of BK channel endocytosis by an RNF-145/EFA-6/ARF-6 molecular pathway
Ragan, Michal; Wang, Zhao-Wen; Chen, Bojun; Niu, Long-Gang; Balsbaugh, Jeremy

BK channels (Slo1) are high-conductance K+ channels gated by voltage and Ca2+. In neurons, BK channels are enriched at presynaptic sites, and serve as an inhibitory regulator of neurotransmitter release. The function and expression of BK channels in vivo depends on many other proteins. In a genetic screen for mutants that suppressed a sluggish phenotype caused by a hyperactive SLO-1, which is C. elegans BK channel, we isolated loss-of-function (lf) mutants of rnf-145, which encodes an E3 ubiquitin ligase. Like slo-1(lf), rnf-145(lf) caused a larger degree of head bending, and increases in the amplitude of evoked postsynaptic currents (ePSCs) and frequency of miniature postsynaptic currents (minis) at the neuromuscular junction (NMJ), suggesting that RNF-145 is required for SLO-1 function in vivo. How might RNF-145 contribute to SLO-1 function? Since ubiquitin E3 ligases generally promote target protein degradation, we reasoned that RNF-145 likely ubiquitinates a negative regulator of SLO-1 rather than SLO-1 itself. To identify the putative negative regulator, we performed mass/specs analyses to detect proteins that are upregulated in rnf-145(lf) mutants compared with wild type. One of the highly upregulated proteins in the mutants was EFA-6, which is a guanine nucleotide exchange factor for the small GTPase ARF-6 (ADP- ribosylation factor 6). It catalyzes the transition of ARF-6 from a GDP-bound inactive state to a GTP-bound active state to promote endocytosis of membrane proteins. We therefore hypothesize that RNF-145 promotes SLO-1 membrane expression by inhibiting its internalization through the EFA-6 and ARF-6 pathway. Consistently, mutants of rnf-145(lf) and slo-1(lf) showed increased ePSC amplitude and mini frequency with non-additive effects in the double mutant, mutants of either efa-6(lf) or arf-6(lf) showed opposite synaptic phenotypes, and both efa-6(lf);slo-1(lf) and arf-6(lf);slo-1(lf) double mutants resembled slo-1(lf). We also found that surface expression of HA-tagged SLO-1 in motor neurons was increased in efa-6(lf) compared with wild type. Our result indicate that SLO-1 surface expression is regulated by an endocytic pathway of RNF-145, EFA-6, and ARF-6.

Neuronal hyperactivation causes an age-dependent decline in associative learning behavior
Noma, Kentaro; Mohri, Mizuho; Mori, Ikue; Tsukada, Yuki; Aleogho, Binta Maria

The neuronal basis of age-dependent behavioral decline is poorly understood. In Caenorhabditis elegans (C. elegans), aging causes a decline in thermotaxis behavior, which reflects animals' ability to associate food availability with their cultivation temperature. Previously, we found that animals fed a lactic acid bacteria, Lb. reuteri, experience less of an age-dependent thermotaxis decline compared to those fed E. coli. Although the thermosensory circuit in C. elegans has been well studied in young animals, the neuronal mechanisms underlying the age- and diet-dependent changes remain unclear. We investigated the neuronal basis using behavioral assays with single neuron-ablated animals and calcium imaging of temperature- evoked neuronal activities. We found that the primary thermosensory circuit for young animals, including AFD sensory neurons and AIY interneurons, was also required for aged animals to perform thermotaxis. However, calcium imaging demonstrated that aging did not drastically change the temperature-evoked neuronal responses of AFD or AIY. By investigating other neurons in the thermosensory circuit, we found that aged animals with AWC ablation maintained a high thermotaxis performance even when fed E. coli. Calcium imaging demonstrated that AWC neurons in aged E. coli-fed animals showed more activation than in young or aged Lb. reuteri-fed animals. These results suggest that aberrant hyperactivation of AWC might disturb the primary thermosensory circuit and lower the thermotaxis ability of E. coli-fed aged animals. Thus, we provide evidence that increased, instead of decreased, neuronal activity can cause a behavioral decline in aged animals in a diet-dependent manner.

Plenary Session 5: Plasticity and Sensory responses

Integration of neuronal activity and synaptic plasticity drives sexually dimorphic learning of pathogenic avoidance

Peedikayil Kurien, Sonu; Haque, Rizwanul; Oren-Suissa, Meital

Sexual selection is instrumental in the development of dimorphic traits that govern the reproductive success of the species. To this end, efficient decision-making strategies have to be developed by critically processing multiple internal and external cues. The underlying mechanisms that govern such sexually dimorphic information processing remain largely unexplored.

Here, we harnessed the multifactorial aversive conditioning paradigm of the pathogenic bacterium Pseudomonas aeruginosa (PA14), which elicits attraction from hermaphrodites of C. elegans initially but aversion upon subsequent exposure (Zhang et al, 2005), indicating learning. Using a choice- based assay which allows us to disentangle sexually dimorphic processing of such complex cues, we observe that, unlike hermaphrodites, males fail to learn PA14 aversion. This dimorphism was found to be independent of differential susceptibility, as irg-4, an immune responder gene, was similarly affected. Such complex decision making is contingent on effective inter-tissue communication. In accordance, several tissue-specific sex-reversal experiments revealed the requirement of the hermaphrodite nervous system but also the gut exclusively in males, suggesting a complex sex-specific regulation. Calcium imaging of AIY, a key interneuron in PA14 learned avoidance unveiled a fine-tuned dimorphic processing of input signals. To gain insight into the molecular entities involved in dimorphic learning, we conducted an unbiased transcriptomics analysis of naïve and trained animals. In line with our previous observations, we notice no dimorphic transcriptome pattern of immune response genes. However, when analyzed for regulators of learning, we identified the possible role of

several neuromodulators. One component, an NPY receptor ortholog, npr-5, was found to be required specifically for male learning. Interestingly, calcium imaging disclosed the role of npr-5 in the naïve processing of pathogenic exposure, leading to dimorphic synaptic output, indicating a possible dimorphic sensory gating mechanism.
Taken together, these results suggest that detrimental environmental cues trigger a series of complex neuronal responses governed by inputs, outputs and sexual identity. Thus, the overall goal of the species fitness is served by highly plastic neuronal processing that is sex-dependent and requires fine- tuned modulation.

PDF-1 modulation of aversion and reward during associative learning
Molina-Garcia, Laura; Colinas Fischer, Susana; Garcia-Minaur-Ortiz, Blanca; Clark, Emma; Truman, Rosie; Benavides-Laconcha, Sergio; Lin, Lucy; Barrios, Arantza

A central goal in neuroscience is to understand how neural circuits integrate conflicting (rewarding and aversive) experiences that need to be behaviourally resolved during learning. To shed light into the molecular and cellular mechanisms underlying this process we have dissected the neuronal circuit that regulates sexual conditioning in C. elegans.
Previously, the Iino lab and us have shown that C. elegans males undergo sexual conditioning, a form of associative learning by which a rewarding experience with mates overrides the behavioural consequences of an aversive association with starvation. Thus, sexual conditioning leads to a switch in behavioural responses to an environmental stimulus from avoidance to attraction.
We also identified the MCM interneurons and PDF-1 neuromodulation as regulators of the sexually conditioned behavioural switch to salt. Here we show that other stimuli such as benzaldehyde can also be sexually conditioned and this is also regulated by the MCMs and PDF-1.
We founda dual role for PDF-1 in the regulation of aversive learning and sexual conditioning in C. elegans. By using a Cre-Lox intersectional strategy we found that PDF signaling encodes both aversion and sexual conditioning by modulating two partially distinct circuits. Within these circuits, we identified the interneurons RIA, AIY and RIM as target cells receiving PDF-1 neuromodulation to drive sexual conditioning.
Furthermore, by removing PDF-1 in specific neurons, while keeping endogenous levels of expression in the rest of the circuit, we identified the MCMs and AVB neurons, as a source of PDF-1 during sexual conditioning and found that the AVBs are activated by mate experience during memory acquisition. Importantly, PDF-1 release from these neurons is not required during aversive learning. Thus, highlighting the importance of source specificity during neuromodulation.
Finally, we measured neuronal activity within the circuit to understand how sensory information is represented after conditioning and PDF-1 neuromodulation. We found PDF-1-dependent activity changes in AIY specifically in sexually conditioned animals. Moreover, we found traces of the aversive memory in sexually conditioned worms, demonstrating that both memories (aversive and rewarding) co-exist during the conflict and compete to express their respective behavioral output.

Nitric oxide produced by gut bacteria modulates foraging behavior through interaction with the oxygen sensing pathway

Kang, WooKyu; Florman, Jeremy T; Thackeray, Andrea; Alkema, Mark J

Animals have evolved intimate symbiotic associations with diverse bacterial communities in their gut. A growing body of evidence suggests that gut microbiota have a wide-range of impacts on animal behavior. However, the molecular basis of how microbiota shape behavior and its host fitness are poorly understood. Here we show Bacillus diet suppresses hyper reversal behavior of unc-2(gf) mutants and modulates foraging behavior of the wild type. We find that intestinal bacteria that produce nitric oxide promotes lawn bordering and food leaving behavior through interaction with the oxygen sensing pathway. Nitric oxide sensing requires the soluble guanylate cyclases GCY-35 and GCY-36 in URX sensory neurons. In contrast to oxygen sensing, nitric oxide signaling is independent of the GMP gated TAX-2/TAX-4 channel. Our data indicate that nitric oxide signaling stimulates cytoplasmic cGMP-dependent protein kinase EGL-4 and regulates G-protein signaling via RGS protein EGL-10. We further show that C. elegans nitric oxide sensing confers a fitness advantage when the animals forage in a patchy food environment. Our findings demonstrate a mechanism of microbiota-host interactions that shapes the host foraging strategy in its natural environment.

Mechanosensory Behaviors of Skin-Penetrating Parasitic Nematodes
Patel, Ruhi; Ly, Cindy; Hallem, Elissa

Skin-penetrating nematodes, including *Strongyloides stercoralis* , infect nearly one billion people worldwide and are major sources of neglected tropical disease. Infective third-stage *Strongyloides* larvae (iL3s) are attracted to heat and host-emitted odorants, behaviors that are thought to increase the probability of host contact. However, whether iL3s detect mechanosensory stimuli, and how mechanosensation enables host seeking and skin penetration remains unclear. We are using *S. stercoralis* and the closely related rat parasite *S. ratti* to answer these questions. We examined the response of iL3s to vibrations that mimic those generated by human locomotion. We found that vibration stimulated an increase in iL3 movement and resulted in robust migration of iL3s toward the vibration source. Vibration also increased nictation and head waving among iL3s, which may facilitate host attachment. To analyze skin-penetration behavior, we observed iL3s on rat skin. The iL3s pushed their heads into the skin and waved their mid- body and tail in the air until the entire animal was inside the skin. The detected rate of penetration for both species was 80–88%. To tease apart the effect of mechanical cues on skin penetration from other cues present on host skin, we developed an*in vitro* assay using alginate hydrogels. Gels with stiffnesses close to human skin were associated with a penetration rate of ~63%, whereas gels that were 10x stiffer did not support penetration. We also identified *S. ratti* homologs of *C. elegans* genes that encode mechanoreceptors. We have generated reporter constructs for some of these genes and observed expression in neurons resembling the touch receptor neurons of*C. elegans* , as well as unidentified head and tail neurons. We are now using CRISPR/Cas9-mediated mutagenesis and chemogenetic neuronal silencing to uncover the mechanosensory genes and circuits that promote host seeking and skin penetration. Our work will illuminate the mechanosensory behaviors of skin-penetrating nematodes and could result in the development of topical anthelmintic creams.

Sensory neuron transcriptomes reveal complex neuron-specific function and regulation of mec-2/Stomatin splicing
Calovich-Benne, Canyon; Norris, Adam; Liang, Xiaoyu

The function and identity of a cell is shaped by transcription factors controlling transcriptional networks, and further shaped by RNA binding proteins controlling post-transcriptional networks. To overcome limitations inherent to analysis of sparse single-cell post-transcriptional data, we leverage the invariant Caenorhabditis elegans cell lineage, isolating thousands of identical neuron types from thousands of isogenic individuals. The resulting deep transcriptomes facilitate splicing network analysis due to increased sequencing depth and uniformity. We focus on mechanosensory touch-neuron splicing regulated by MEC-8/RBPMS. We identify a small MEC-8-regulated network, where MEC-8 establishes touch-neuron isoforms differing from default isoforms found in other cells. MEC-8 establishes the canonical long mec-2/Stomatin isoform in touch neurons, but surprisingly the non- canonical short isoform predominates in other neurons, including olfactory neurons, and mec-2 is required for olfaction. Forced endogenous isoform- specific expression reveals that the short isoform functions in olfaction but not mechanosensation. The long isoform is functional in both processes. Remarkably, restoring the long isoform completely rescues mec-8 mutant mechanosensation, indicating a single MEC-8 touch-neuron target is phenotypically relevant. Within the long isoform we identify a cassette exon further diversifying mec-2 into long/extra-long isoforms. Neither is sufficient for mechanosensation. Both are simultaneously required, likely functioning as heteromers to mediate mechanosensation.

Viral infection in C. elegans promotes sleep, which is necessary for survival and energy maintenance
Iannacone, Michael; Raizen, David; van der Linden, Alexander

Acute illness causes sickness behavior, which consists of reduced movement, anorexia, social withdrawal, and sleep. In*C. elegans* , cellular stressors such as heat shock and ultraviolet radiation (UV) cause stress-induced sleep (SIS). While studies of worm SIS inform our understanding of the behavioral response to acute illness, they have questionable relevance to chronic human ailments such as HIV infection, long COVID syndrome, and multiple sclerosis. The severe fatigue observed in these chronic diseases is a large source of disability in our society yet is not understood. We therefore sought to develop a model for studying sickness behavior during chronic illness. For this purpose, we used infection with the Orsay virus. The Orsay virus exclusively infects intestinal cells yet strongly affects behavior, indicating a gut-nervous system axis for communication. Similar to UV and heat, mutations that affected development of the ALA neuron and processing of neuropeptides reduced sleep in Orsay infected animals. ALA-defective animals had decreased survival, suggesting a benefit of sleep to survival during chronic sickness. Overexpression of the somnogenic FLP-13 neuropeptides increased the survival of infected ALA-defective animals, suggesting that the loss of sleep was specifically responsible for decreased survival. The survival benefit of sleeping animals was not explained by a difference in viral replication levels or in the transcriptional intracellular pathogen response; rather, it is better explained by resiliency of the animals. Infection with Orsay caused a decrease in whole animal ATP levels, and this decrease was more severe in ALA-defective animals, suggesting that sleep is required to preserve energetic supplies. Viral infection was accompanied by proliferation of bacteria in the animals, and lifespan was mildly

extended in virus-infected animals grown on dead bacteria, suggesting that bacterial superinfection is partially responsible for virus-induced lethality. These findings suggest that sleep is beneficial for recovery from infection due to its effect on energetics. This model presents an opportunity to explore the protective role of sleep in host-pathogen interactions.

Insulin-like peptides control the dauer exit developmental decision in C. elegans
Zhang, Mark; Sternberg, Paul

Under unfavorable environmental circumstances, C. elegans will enter the alternative diapause state known as dauer in order to protect itself against environmental stress. When conditions sufficiently improve by way of increased food and decreased crowdedness, C. elegans exit the dauer state and return to reproductive growth. The neurobiological and genetic mechanisms that underpin the dauer exit developmental decision
remain sparsely studied. We demonstrate that neuropeptides are collectively required for the dauer exit decision because mutants defective for neuropeptide processing or secretion exhibit severe dauer exit defects. To determine which specific neuropeptides regulate the dauer exit decision, we leveraged single-cell RNA-sequencing (scRNA-seq) data and examined which neuropeptide genes were specifically expressed in ASJ, a chemosensory neuron previously shown using ablation studies to be essential for dauer exit. Using an endogenously relevant dauer exit assay, we demonstrate
that ins-6 and daf-28, two insulin-like peptide genes predicted by scRNA-seq to be enriched in ASJ, are indeed bona fide regulators of the dauer exit decision. We show that during dauer exit, ins-6 and daf-28 are both transcriptionally upregulated in ASJ, and INS-6 is secreted throughout the body cavity. To examine how ASJ processes sensory cues, we performed calcium imaging of ASJ and found that ASJ responds to food but not pheromone (a chemical indicator of crowdedness). Given that ins-6 and daf-28 expression in ASJ reflects environmental conditions in terms of both food and pheromone, these results suggest that ASJ receives input from other pheromone-sensing neurons to calculate an internal metric of food and pheromone concentrations. In sum, we show how insulin-like peptides regulate the dauer exit developmental decision by acting as signaling molecules secreted by environmentally sensitive chemosensory neurons and targeted to downstream target tissues.

The C. elegans chemosensory system decodes complex blends of microbial metabolites to distinguish benign and pathogenic bacteria
Brissette, Benjamin; Ringstad, Niels

Animals use chemosensation to navigate environments. Many ethologically relevant chemical stimuli, for example food odors, are complex blends, not monomolecular. How chemical mixtures are decoded by the sensory nervous system to control specific behaviors remains poorly understood. The roundworm C. elegans is an excellent model in which to study this transformation. We find that C. elegans uses its chemosensory system to rapidly and robustly distinguish nutritive E. coli bacteria and a dangerous pathogen, E. faecalis. To identify the chemical features used by C. elegans to distinguish these microbes, we profiled metabolites produced by E. faecalis and E. coli and determined a metabolic 'fingerprint' of each species. In parallel, we measured microbe-specific responses to microbial metabolites. Our results

suggest that microbe-identity is encoded by a population of chemosensory neurons, which are likely sensing different metabolites.
To identify components of a downstream circuit that decodes sensory neuron activity to generate microbe-specific behaviors, we used the calcium integrator CaMPARI to screen for interneurons with microbe-specific responses. We found that AIB interneurons specifically respond to cues provided by E. coli bacteria, suggesting that AIBs integrate inputs from sensory neurons tuned to E. coli-specific metabolites. Notably, silencing AIBs impairs chemotaxis to E. coli. Together, these data support a model in which AIB plays a critical role in generating specific behavioral responses to nutritive and pathogenic microbes by decoding complex patterns of sensory neuron activity elicited by microbial metabolites.

Plenary Session 6: Aging, Neural Diseases and Regeneration

hnRNPQ/hrpr-1 and RTN/ret-1 role in splicing and neurodegeneration.
La Rocca, Federica; Santonicola, Pamela; Cieri, Federica; Zampi, Giuseppina; Nizzardo, Monica; Di Schiavi, Elia; Corti, Stefania

A correct splicing of mRNA is globally required in all cells, but neurons seem highly sensitive to perturbations with numerous neurological diseases caused by splicing defects, including spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS), and dementias. However, why neurons are more affected to splicing alterations and which step of RNA processing is impaired in these diseases is still debated. We are investigating the molecular mechanisms underlying the neurodegeneration caused by splicing defects in SMA. SMA is a motor neurons (MNs) specific disorder caused by mutations in the Survival Motor Neuron (*Smn1*) gene. In our previous work, RNA-sequencing of induced pluripotent cell-derived motor neurons (iPSC-MNs) from SMA patients and healthy people allowed the identification of differentially expressed/spliced genes, enriched in RNA motif 7 (CAAAAAG). This motif is specifically bound by hnRNPQ, a spliceosomal component, physically interacting with SMN in the SMN complex. *hrpr-1* and *smn-1* are the *C. elegans* homologs of *hnRNPQ* and *Smn1* . We determined that *hrpr-1(KO)* animals show pleiotropic phenotypes similar to the one observed in *smn-1(KO)* , as larval arrest, a severe decrease in lifespan and locomotion defects. *hrpr-1* mutants also show a reduction in visible D-type MNs and axonal defects, suggesting a role in neuron survival. We demonstrated that *hrpr-1* and *smn-1* genetically interact in MNs, where the overexpression of *hrpr-1* rescues the neurodegeneration caused by the MNs-specific silencing of *smn-1.* Since *hrpr-1* is involved in the alternative splicing of many neuronal genes, we investigated the role in *smn-1* pathway of a well-known *hrpr-1* target, *ret-1/RTN* . We determined that in *smn-1(KO)* and *hrpr-1(RNAi)* animals the splicing pattern of *ret-1* is altered. We also demonstrated that the rescue of MNs degeneration obtained after *hrpr-1* overexpression in *smn-1(MNs RNAi)* animals, is partially mediated by *ret-1.* Interestingly the role of *ret-1/RTN* in neurodegeneration and SMA is conserved between species since we observed that *RTN* transcription levels are altered in SMA mice spinal cord and in iPSC-MNs from SMA patients. These data identify, for the first time, a neuroprotective role of *hrpr-1* and *ret-1* in MNs and their possible role as potential new therapeutic targets.

Histidine phosphorylation-mediated signal transduction regulates axon regeneration in C. elegans

Sakai, Yoshiki; Hanafusa, Hiroshi; Hisamoto, Naoki; Matsumoto, Kunihiro

Protein phosphorylation is one of the most important post-translational modifications that regulate and diversify protein functions. In animals, phosphorylation of serine, threonine, and tyrosine are well characterized, but relatively little is known about phosphorylation of histidine (pHis). Previous studies with cultured mammalian cells have shown that His-phosphorylation is regulated by the His-kinase NDPK and the pHis-phosphatase PHPT1. However, their physiological functions in vivo remain largely unknown. To understand the*in vivo* role of His-phosphorylation in animals, we used the nematode *C. elegans* as a model organism. Here we show that His-phosphorylation plays an inhibitory role in axon regeneration, an evolutionarily conserved neuronal response in which neurons regenerate damaged axons to restore their function. We found that the*C. elegans* His- kinase NDK-1/NDPK inhibits axon regeneration by phosphorylating GPB-1 Gβ at His-266, while the pHis-phosphatase PHIP-1/PHPT1 promotes axon regeneration by counteracting this phosphorylation. Previous studies have shown that Gβ His-phosphorylation activates Gα in a receptor-independent manner. Consistently, our genetic analysis suggests that GPB-1 His-phosphorylation inhibits axon regeneration by activating GOA-1 Goα, a negative regulator of axon regeneration. Furthermore, we found that the autophagy-activating kinase UNC-51/ULK phosphorylates PHIP-1, and this phosphorylation is essential for PHIP-1-mediated axon regeneration. These results suggest that during axon regeneration, UNC-51 activates PHIP-1, and PHIP-1 dephosphorylates GPB-1, thereby inactivating GOA-1 signaling and promoting axon regeneration. This finding provides one example of how reversible His-phosphorylation regulates biological functions in living animals.

Imaging age-related alterations in neuronal dynamics in C. elegans

Gabel, Christopher; Wirak, Gregory; Connor, Christopher

In the aging brain, many of the alterations underlying cognitive and behavioral decline remain opaque. Specifically, how normal aging and neurodegenerative states change neuronal connectivity and circuit function on the cellular level remains largely unknown. C. elegans offers a powerful model for aging research, and with its simple, well-studied nervous system, presents a unique opportunity to measure and understand the system-wide functional alterations in neuronal senescence. Employing volumetric fluorescence microscopy, we have performed multi-neuronal functional imaging across the aged C. elegans nervous system. We measure a progressive age-associated breakdown in system-wide organization and temporal continuity that begins in mid-adulthood. Interestingly, we also measure a progressive increase in bouts of global neuronal quiescence with age that appear to be neuronally similar to sleep states observed during development. At single-cell resolution, aging results in a shift in neuronal activity toward higher frequency dynamics and a specific loss of anti-correlated activity (i.e. inhibitory signaling) between neuron pairs. Importantly, the degree of positively correlated activity (i.e. excitatory signaling) remains unchanged resulting in an overall disruption of the systems excitatory/inhibitory balance with age. These effects are recapitulated by mechanisms known to alter GABAergic signaling. During development, calcium channel subunit UNC-2/CaV2 activity is known to trigger the removal of inhibitory GABAergic synapses in motor neurons through a CED-4 dependent pathway. Accordingly, we

find that young adult animals with a gain of function unc-2 mutation exhibit neuronal dynamics and behavior similar to that of aged animals, while ced-4 loss-of- function mutation limits neuronal decline with age. Likewise, we find that increases/decreases in inhibitory GABA signaling also partially ameliorate/accelerate the aging effects. Disruption of the insulin signaling pathway through daf-2 mutation, that is known to increase longevity, also partially mitigates the effects of aging on neuronal dynamics. Data from mammals are consistent with our findings, suggesting a conserved shift in the balance of excitatory/inhibitory signaling with age that leads to breakdown in global neuronal dynamics and functional decline. Our results here suggest that it is specifically a loss of inhibitory signaling that drive this disruption in excitatory/inhibitory balance with age.

The metalloprotease ADM-4/ADAM17 promotes axonal repair
Ho, Xue Yan; Coakley, Sean; Amor, Rumelo; Anggono, Victor; Hilliard, Massimo

Axonal damage, such as in nerve injuries, interrupts the communication between a neuron and its target tissue. Functional recovery is achieved when the regenerating axon re-innervates its original target tissue. However, intervention to repair such damage is still not achievable. Axonal fusion is an efficient means of repair following axonal transection, whereby the proximal axon, still attached to the cell body, regrows and re-establishes membrane and cytoplasmic continuity with its own separated axonal fragment, restoring neuronal function. The molecular mechanisms of this process are not fully elucidated. In C. elegans, a key function has been established for the fusogen EFF-1, which mediates the merging of the plasma membranes of the two separated fragments. Using a candidate gene approach, and the C. elegans PLM mechanosensory neurons as a model system, we identified ADM-4 as a key regulator of EFF-1-mediated axonal fusion. ADM-4 is a member of the ADAM (A Disintegrin and Metalloprotease) family, and ortholog of the human ADAM17/TACE (Tumor necrosis factor Alpha-Converting Enzyme). adm-4 loss-of-function leads to a severe reduction of axonal fusion in PLM neurons without affecting axonal regrowth. We demonstrate that ADM-4 regulates this process in a cell-autonomous fashion and observe dynamic changes in the subcellular localisation of ADM-4 after axotomy. Furthermore, overexpression of ADM-4 selectively in the PLM neurons is sufficient to enhance axonal fusion in wild-type animals. We have previously shown that phosphatidylserine (PS) exposure on the damaged axon functions as a “save-me” signal to promote fusion. Our recent data show that putative PS-binding sites in ADM-4, as well as its metalloprotease activity, are essential for its function. We propose that PS exposure triggered by injury binds ADM-4 and activate its proteolytic function. Finally, biochemical analysis reveals that ADM-4 binds and stabilises EFF-1 to promote axonal fusion. Our results uncover an essential function for ADM-4 in promoting axonal fusion and set the foundation for the design of novel therapeutics for nerve injuries.

An intestinal sphingolipid promotes neuronal health across generations
Wang, Wenyue; Pocock, Roger

Maternal diet and environment influence the neuronal health of offspring. However, the underlying mechanisms of how maternal nutrition affects neurodegeneration are unclear. Here, we report that maternal diet controls intestinal sphingolipid metabolism to prevent adult-onset axon degeneration for two generations. Ursolic acid (UA) is a natural compound

found in fruit and is part of a healthy diet. We found that UA protects against axon degeneration intergenerationally through increased maternal provisioning of an intestinal sphingolipid. Consistent with this result, sphingolipid supplementation mimics UA function in suppressing axon degeneration intergenerationally. Mechanistically, UA enhances sphingolipid production by upregulating the expression of an acid ceramidase in the intestine. The sphingolipid is then transferred from the maternal intestine to oocytes to promote intergenerational neuroprotection. Overall, our results demonstrate that UA supplementation in the hermaphrodite diet prevents axon degeneration over multiple generations and reveals a novel role of diet-mediated intestinal sphingolipid metabolism in neuronal health.

Anticipatory activation of the UPRER by pathogen-associated odour
A de Souza, Evandro; Taylor, Rebecca; A Thompson, Maximilian

C. elegans live in pathogen rich environments in the wild and are likely to encounter a huge variety of stresses and pathogens. We wondered whether worms could activate stress responses in an anticipatory manner based upon their perception of the external environment. Our lab is particularly interested in the UPRER and the UPRER is known to be activated by pathogenic Pseudomonas spp. We therefore wondered whether the odour of pathogenic bacteria can induce the UPRER. To this end we undertook a mini screen of various odourant molecules secreted by Pseudomonas spp. Excitingly, we identified two structurally-related odourant molecule which induced the UPRER. The best tolerated of these molecules was 1-undecene, which we investigated further.
Here, we present evidence that 1-undecene induces xbp-1 splicing in neurons, leading to the activation of the cell non-autonomousUPRER in the intestine via unc-13. Curiously, this non-autonomous activation is independent of tyramine signaling, previously shown to be required for non- autonomous UPRER activation in a genetic model of xbp-1s neuronal overexpression. Importantly, this activation of the UPRER by the input of chemosensory information was sufficient to extend lifespan and protect against PolyQ accumulation. Our work suggests that the cell non-autonomous UPR allows animals to activate the UPRER in an anticipatory manner in response to perception of a pathogen, pre-empting the arrival of proteostatic stress. Additionally, the activation of xbp-1 in neurons by chemosensation implies that the input of chemosensory information may be a novel way of manipulating stress responses in neurons more broadly.

Impact of Traumatic Brain Injury on sensory neural function, behavior, and neural structure in C. elegans
White, Hamilton; Albrecht, Dirk

Traumatic brain injury (TBI) causes polymodal trauma leading to persistent changes in brain function, behavior, cellular structure, and is a known risk factor for neurodegenerative disease. Current injury models correlate the presence and duration of injury conditions with animal behavior, but they do not reveal underlying effects on brain function at the cellular and subcellular scale. To identify underlying mechanisms relating acute brain injury with functional outcomes, we sought to develop a reliable, scalable TBI model in C. elegans to directly observe injury progression at behavioral, neurofunctional and structural levels, both immediately and over hours to days. Previously, ultrasonic shock waves and vortex-induced blunt force trauma

caused paralysis in thrashing animals, but with broad population variability. We investigated ultrasonic cavitation as a repeatable and titratable TBI induction method using a bath sonicator modified for precise, sub-second timing control. Injury levels were assessed by body morphology, movement, neural structure, and stimulated neural responses before and after sonication for a range of duration, repetition and housing formats.
Video recordings during sonication revealed that animals near a rigid surface were injured in a dose-dependent manner, adopting a straightened, stationary posture, whereas those in bulk liquid were relatively unharmed. Animals in liquid tubes or wells exhibited a large population variability, consistent with prior reports, whereas those in microfluidic chambers were more uniformly injured. At high injury levels in the microfluidic format (multi-second sonication durations), we found animals were paralyzed with no muscle activity and disrupted neural structure. Moderate injury levels were characterized by body twitches, random bursts of muscle cell activity, and diminished chemosensory responses (stimulus-evoked neural activity and chemotaxis behavior). Mild injury modulated sensory sensitivity in the absence of behavioral and muscle cell activity defects. Post-injury behavioral responses were partially determined by feeding state and age. Repeated assessment of neural function of up to 12 hours allowed examination of neurofunctional recovery. Overall, sonication-induced TBI provides repeatable assays for real-time, in vivo recording of neuronal structure, function, and behavior, before and after single or repeated injury, enabling further study on injury mechanisms, progression, and potential therapies to minimize damage and enhance recovery.

Semaphorin signaling restricts neuronal regeneration.
Harreguy, Maria Belen; Haspel, Gal; Tanvir, Zainab; Sha, Esha; Simprevil, Blandine; Tran, Tracy

Extracellular signaling proteins that mediate neuronal growth cone guidance during development are well positioned to be involved in neuronal regeneration and recovery from injury. Semaphorins and their receptors, the plexins, are a family of highly conserved proteins involved in axon pathfinding and synapse formation during development. The Caenorhabditis elegans genome encodes for only 3 semaphorins and 2 plexin receptors, compared to 20 semaphorins and 9 plexins, in the mammalian nervous system. The transmembrane semaphorins, SMP-1 and SMP-2, signal through their receptor, PLX-1, while the secreted semaphorin, MAB-20, signals through PLX-2. Here, we took advantage of the small number of plexins in C. elegans, and the capability to precisely disconnect single neurites in intact animals using femtosecond laser microsurgery, to investigate the role of semaphorin signaling in neuroregeneration in vivo. Using co-expression with NeuroPAL for precise identification, we described the neuronal expression patterns of the plexin promoters in the ventral nerve cord. plx-1p induced expression in the body wall muscle and in two motoneurons at the end of the tail, DA8 and DA9, while plx-2p induced expression in five motoneuron classes in the VNC, mostly DA and AS, which extend commissures that were the targets for microsurgery. Further, we investigated the regrowth andreconnection of motoneuron neurites, and the recovery of locomotion behavior following precise laser microsurgery in plexin-knockout animals. Regrowth and reconnection were more prevalent in the absence of each plexin, while recovery of locomotion surpassed regeneration in all genotypes. Our results suggest that the secreted and membrane-bound semaphorin signaling pathways both restrict regeneration but in distinct processes that likely include spatial specificity and recurrent signals.

Poster session I

1. **(-)-Gossypol inhibition of musashi-mediated forgetting improves memory and age-dependent memory decline in Caenorhabditis elegans**
Mastrandreas, Pavlina

Musashi RNA-binding proteins retain a pivotal role in stem cell maintenance, tumorigenesis, and nervous system development. Recently, we showed in C. elegans that MSI1 actively promotes forgetting upon associative learning via a 3' UTR-dependent translational repression of the Arp2/3 actin branching complex. Here, we investigated the evolutionary conserved role of MSI proteins and their druggability in the modulation of forgetting. Expression of human MSI1 and MSI2 under the endogenous musashi promoter fully reversed the decreased forgetting of msi-1(lf) worms. Furthermore, pharmacological inhibition of MSI1 and MSI2 activity using (-)-gossypol resulted in decreased forgetting, without causing locomotor, chemotactic or learning deficits. The effect of (-)-gossypol on memory is likely mediated via musashi as no drug effect was observed in msi-1(lf) treated worms. Moreover, using Western blotting and confocal microscopy we found no changes in MSI-1 protein abundance following (-)-gossypol treatment; suggesting that the protein remains functional and is most likely inhibited upon drug exposure. Additionally, (-)-gossypol suppressed the previously seen rescue of the msi-1(lf) phenotype in worms expressing human MSI1 specifically in the AVA neuron; indicating that (-)-gossypol likely regulates the musashi pathway in a tissue-specific manner. Finally, treating aged worms with (-)-gossypol reversed physiological age-dependent memory decline. Taken together, our findings indicate that pharmacological inhibition of musashi might represent a promising novel drug approach for the treatment of memory deficits.

2. **A microfluidic chip to expose C. elegans chemosensory neurons to multiple chemicals sequentially and simultaneously image IFT proteins at single-molecule level**
Haasnoot, Guus; Bruggeman, Christine; Peterman, Erwin

The nematode C. elegans has a relatively simple nervous system of only 302 neurons, 32 of which are specialized neurons able to sense the chemical environment. These neurons can sense attractive and/or repulsive ques and use this information to change their behaviour accordingly, a very useful feature to aid survival. Two pairs of chemosensory neurons are located in the tail of the worm and are denoted PHA and PHB. Through an opening in the cuticle, the ciliated ends of these neurons are exposed to the environment. Therefore, they are able to sense the surrounding chemical composition through receptors located in the membrane. Inside the cilium, intraflagellar transport (IFT) maintains the structure of the organelle and transports sensory proteins to the tip and back. However, how exactly this transport system is regulated and how its regulation relates to neuronal signalling is not yet known. To investigate this, we use a microfluidic chip to stimulate the tail of the worm with aversive chemicals at high temporal resolution. We image the change in calcium concentration in our neurons of interest, which is a measure for neuronal activity, by using a calcium indicator. Moreover, we study different IFT components which have been fluorescently labelled. With this method we have observed an interesting change in IFT dynamics and

axoneme structure as a result of exposure to various aversive chemicals. To obtain more mechanistic insight into this change in IFT dynamics we recently developed a method to study the IFT components on the single-molecule level while exposing the worm with an aversive chemical. In the future we would also like to expose the worm to multiple chemicals sequentially. We are therefore in the process of altering the current microfluidic chip design to expand its capabilities.

3. A reference dataset of C. elegans whole-brain neural activity

Bubnis, Gregory; Borchardt, Jackson; Kato, Saul; Xu, Yifan

The compact nervous system of *C. elegans* exhibits complex dynamical patterns of neuronal network activity. Understanding how these patterns are generated, their structure, and how they produce complex behavior is a major goal of systems and computational neuroscience. A substantial corpus of neuronal network activity data would pave the way for diverse, statistically powerful analyses of this exquisite dynamical system.
We present a large, high quality collection of whole brain activity recordings for community use. Using the OH16230 worm strain, which combines the NeuroPAL transgenic payload with pan-neuronal nuclear-localized GCaMP6s, we imaged paralyzed young adults loaded into a microfluidic channel using two different commercially available confocal spinning disk microscopes. The dataset comprises 50 worms, each with 150-190 segmented neurons from the head and, in some cases, part of the ventral nerve cord.
Several innovations enabled the acquisition of high quality volumetric datasets, including scripts to acquire in alternating recording modes, developed on the open source microscopy package Micro-Manager; methods of reducing translation of animals within microfluidic channels; and the integration of a fast pan-chromatic volumetric scan mode to produce a dataset for neural segmentation more resistant to motion artifacts.
For each recording, we acquired a multi-channel, NeuroPAL volume followed immediately by a 4-15 minute GCaMP timelapse recording. By cross referencing multi-channel color data to the NeuroPAL reference atlas, we manually ID'ed 50-100 neurons in each recording. We apply our pipeline for machine-assisted analysis including extraction, tracking, ID and human curation to this dataset.
The dataset should be immediately useful for benchmarking automated time series extraction and neuronal ID approaches. It should also be useful for computational analysis and theoretical neuroscientific investigations, including detailed characterization of the neuronal state space and study of the dynamical substrate of the production of behaviors.
We envision that the raw data, extracted time-series, and analysis code can be a seed for a larger open community resource of reference neural imaging datasets collected under different experimental and biological parameters.

4. Acute response of hydrogen sulfide in C.elegans

Pu, Longjun; Wang, Jing; Chen, Changchun

Hydrogen sulfide (H2S) is a natural product in human cells. Endogenous H2S has been emerging as an important signal molecule in many physiological processes. Exogenous H2S has been shown to effect physiology beneficially or adversely based on the concentration of exposure. Although it is well known that acute exposure to high concentration of H2S has

immediate toxicity to human, the sensing machinery and its molecular mechanisms are still in debates. Here to investigate the acute sensation of H2S, we expose the genetically tractable nematode Caenorhabditis elegans acutely to atmospheres containing H2S. we found C. elegans can generate fast and strong responses to acute H2S exposure in a dosage-dependent manner. This fast response to acute H2S can be modulated by HIF-1 signaling, oxygen sensing pathway and mitochondrial ROS in C. elegans. In addition, we found that many ciliary mutants fail to response to H2S. Our ongoing efforts are focused on fully understanding how these signaling pathways modulate acute H2S sensing. We believe that our discoveries have the potential to gain molecular insight into molecular and neural circuit bases of acute H2S responses.

5. **Adenosine receptors as potential therapeutic targets in movement disorders/epilepsy caused by GNAO1 de novo mutations Martinelli, Simone;** Di Rocco, Martina; Lanza, Enrico; Tosato, Federica; Follo, Francesca Carmen; Friedman, Jennifer; Caprini, Davide; Martire, Alberto; Folli, Viola; Di Schiavi, Elia; Galosi, Serena; Leuzzi, Vincenzo

Dominant mutations in the GNAO1 gene underlie a severe neurological condition characterized by hyperkinetic movement disorders, drug-resistant seizure, developmental delay, and cognitive decline, with infantile/childhood onset. GNAO1 encodes the α-subunit of an inhibitory GTP/GDP-binding protein regulating ion channel activity and neurotransmitter release. The pathogenic mechanisms underlying GNAO1-related disorders remain largely elusive and to date there are no effective therapies. Here we generated CRISPR-Cas9-engineered C. elegans strains harboring multiple pathogenic variants (i.e., p.S47G, p.R209H, and p.E246K) in goa-1, the C. elegans orthologue of GNAO1. An additional strain carrying the p.A221D change was already available at the CGC. Like null mutants, homozygous animals showed increased egg laying and were hypersensitive to aldicarb, an inhibitor of acetylcholinesterase, suggesting excessive neurotransmitter release by different classes of motor neurons. Automated analysis of C. elegans locomotion indicated that goa-1 mutants move faster than control animals, with more frequent body bends and a higher reversal rate, and display uncoordinated locomotion. Phenotypic profiling of heterozygous nematodes revealed a loss-of-function or a dominant-negative behavior of the pathogenic variants. A pilot drug screening performed with compounds targeting G-protein coupled receptors indicated that the hyperactive motor behavior of goa-1 mutants as well as of animals lacking goa-1 is mitigated by caffeine, an antagonist of adenosine A1 and A2A receptors. This effect is mimicked by istradefylline, a selective FDA-approved A2A receptor antagonist used in the treatment of Parkinson's disease, indicating that both drugs act as bypass suppressors.
Conversely, the selective A1 receptor agonist CPA increased the number of reversals per minute of wild-type nematodes. Competitive assays confirmed that caffeine act via adenosine receptor antagonism. Overall, our findings establish C. elegans as an efficient drug-screening platform for GNAO1- related disorders and highlight the potential role of adenosine receptor antagonists in controlling dyskinesia.
Keywords: movement disorders, epilepsy, goa-1/GNAO1, caffeine, adenosine receptors, bypass suppressors.

6. **Altered gravity force hinders proper development of dendritic structures in a touch sensory neuron PVD in C. elegans**

Moon, Je-Hyun; Lee, Jin

Space flight has shown that altering gravity can affect many biological processes including muscle and bone development. However, gravity's effect on neuronal development is not clear. Previously, we showed that hypergravity affects axonal development of DD/VD motor neurons. Here, we will investigate the effects of altering gravity on neuron dendrite development by observing the PVD neuron, a harsh touch sensory neuron, in C. elegans in different gravity conditions. The PVD sensory neuron develops post-embryonically, and by adulthood displays intricately organized and non-overlapping dendrites spanning most of the body length. To investigate whether PVD development is normal in altered gravity, we exposed C. elegans to 100G hypergravity in a centrifuge from egg to young adult. We identified hypergravity-induced abnormal structures in the PVD neuron, particularly 4° branch defects, including "L" and "T" shape 4° branch defects. To determine if there is a critical period during PVD neuron development that hypergravity acts on, we exposed C. elegans to different gravity time frames. We show that PVD 4° branches are affected by 100G hypergravity during the 48-72hr time frame which is consistent with the fact that 4° branches develop during the L4 stage. Since basement membrane protein UNC-52/perlecan is known for its fundamental role for patterning PVD 4° branches and increased "L" and "T" shape 4° branch defects, we exposed unc-52 mutants to hypergravity.
Results show that unc-52 mutants can suppress hypergravity-induced PVD "L" and "T" shape 4° branch defects.
We also performed C. elegans transcriptome analysis to identify gene expression pattern changes in 100G hypergravity. This work will offer a fundamental foundation for elucidating how hypergravity alters neuron dendrite development in C. elegans. We are also testing PVD development in real microgravity aboard the International Space Station, and simulated microgravity in a 3D clinostat in the laboratory.

7. **An improved C. elegans model of Alzheimer's Disease to monitor neuronal signalling activity**

Bajuszova, Viktoria; Cohen, Netta; van Oosten-Hawle, Patricija

Alzheimer's disease (AD) is the most prevalent form of neurodegenerative diseases, characterised by the presence of Aβ plaques and neurofibrillary tangles mediating memory impairments due to loss of neurons and impairments in neuronal plasticity. One of the earliest symptoms of AD is olfactory dysfunction. The olfactory response requires glutamatergic transmission and growing evidence suggests that alterations in glutamatergic neuronal signalling caused by the toxic effects of Aβ are underlying causes of the loss of neurons and neuronal synaptic plasticity during the progression of the disease. We are building a new C. elegans AD model that allows to measure alterations in neuronal signalling, using glutamate and calcium sensors expressed in glutamatergic neurons. This will enable us to identify potent neuronal modifiers as well as determine which glutamatergic neuronal circuit succumbs first to the toxic effects of human Aβ expression in C. elegans neurons.

8. **An Olfactory-Interneuron Circuit That Drives Stress-Induced Avoidance Behavior in C. elegans**

Chen, Yen Ju; Chang, En-Ni; Pan, Chun Liang

Learned aversion is critical for animal survival by minimizing the risk of exposure to environmental hazards or predators. Internal states are powerful influences on avoidance behavior and are known to induce aversive associative learning. However, the neural circuits and signaling mechanisms underlying stress-induced aversion are incompletely understood. Our recent work characterized serotonergic and dopaminergic circuits for bacterial aversion of C. elegans induced by mitochondrial stress. Here, we further show that olfactory neurons and the glutamatergic signaling from AWC olfactory neurons regulate stress-induced avoidance behavior via the AIY interneurons. The live AIY response shows that the AIY activity induced by olfaction. Ablation of the AIZ triggered bacterial avoidance regardless of the stress. RIA and AIB interneurons, which are part of the circuits for avoiding pathogenic bacteria, are dispensable for stress-induced bacterial aversion. Finally, we showed that the head motor neurons SMDD/V were essential, and ablation or inhibition of these neurons altered the worm's locomotion that likely contributed to reduced avoidance. These findings substantiate our understanding of the circuit mechanisms that underlie learned bacterial avoidance triggered by mitochondrial insults.
(supported by the National Health Research Institutes, NHRI-EX111-11134NI; and the Ministry of Science and Technology, MOST 109-2320-B-002-019- MY3)

9. **An Open Source Pipeline and Interactive Application for Quantification, Visualization, and Curation of Whole-Brain Neural Calcium Activity from Volumetric Microscopy of C. Elegans**
Borchardt, Jackson; Ban, Steven; Bubnis, Greg; Dunn, Raymond; Kato, Saul

Live volumetric microscopy of model organisms engineered to neuronally express genetically encoded calcium indicators has become a widespread technique to record neural network activity at single-cell resolution. As with many new biotechnological techniques, C. elegans researchers have pioneered this field, yet there is still a lack of mature, robust, easy-to-use, software tools for high quality extraction of neural activity time series from C. elegans volumetric whole-brain recordings. High-accuracy automation of the time series extraction process remains an unachieved goal; therefore interactive visualization and curation is still required to produce high quality datasets. We have developed a flexible, open source pipeline for automated and curated time series extraction, written in Python and usable by scientists without the need for coding. The core data analysis algorithms are robust, interpretable, and effective, and our pipeline architecture allows modular substitution of other algorithms and computational elements. We leverage the open-source packages scikit-image and OpenCV for automated segmentation, tracking, and time series extraction of neurons. Additionally, we have built an interactive GUI application for real-time 4D visualization, annotation, and curation of volumetric recordings, based on the multi- dimensional image viewing application napari. We test our pipeline using reference datasets from prior whole-brain imaging publications and new spinning-disk confocal recording datasets and find that our pipeline achieves a high degree of accuracy while maintaining computational efficiency.

10. **An open-source script to determine the chemotaxis score of a population at user-defined time intervals**
Hodge, Francesca; Ilett, Tom; Cohen, Netta; van Oosten-Hawle, Patricija

In order to survive Caenorhabditis elegans must be able to effectively seek out food, avoid harmful substances, including pathogens and find mating partners. To sense the environment, C. elegans use neuronal ciliae. These attraction and avoidance-based behaviours are commonly known as chemotaxis behaviours. Several powerful tools exist to track C. elegans chemotaxis. There are, however, some restrictions in many existing software such as a requirement of specific specialised equipment. Additionally, the incorporation of object tracking, although highly powerful, may not always be of requirement and relying on a high frame rate video often results in large files. In instances where an overview of the population is required it may not be necessary to utilise single-worm tracking, omitting this object tracking allows for larger time intervals between images which can allow for a longer experimental timespan. Here we describe a method that allows automatic image analysis to quantify chemotaxis behaviour over time. Using chemotaxis-deficient C. elegans mutants and neurodegenerative disease models, we provide evidence on the value of this method which allows for a flexible setup design and user-defined time intervals. As this analysis is based on pixel values rather than object detection, collisions do not result in a loss of animals and can be flexible to any imaging system which is able to generate images with enough contrast to be binarized. Once fully developed and validated this analysis script will be made available for open-source download.

11. Automatic analysis of feeding behaviour for tracking the evolution of novel feeding states in the predatory nematode Pristionchus pacificus

Eren, Güniz Göze; Lightfoot, James W.; Scholz, Monika

Neuroscientists have long studied the molecular and neural mechanisms of behavioural states that influence how sensory information is processed and how behaviours are generated.These persistent states are often controlled by neuromodulatory systems that typically influence the activity of neurons distributed throughout many brain regions. Global working mechanisms of neuromodulators poses challenges for understanding their mode of action. Caenorhabditis elegans has been a favoured model to study how internal and external cues are integrated to give rise to behavioural states due to the simplicity of the nematode nervous system. Recently, another nematode, Pristionchus pacificus has been established as a ‘satellite’ model organism to
C. elegans to explore its evolutionarily divergent developmental and behavioural features. Due to the presence of teeth-like denticles, P. pacificus displays predatory and cannibalistic behaviours in addition to its bacterial feeding. A neuromodulator, serotonin, is a key molecule that regulates predatory feeding behaviours in P. pacificus. Here we are investigating the influence of serotonin further on theexploration and exploitation foraging states in P. pacificus to study how predatory feeding dynamics are regulated and how they may have evolved. In order to study P. pacificus feeding dynamics, we have begun to develop an automated predatory behavioural method based on tracking of the P. pacificus pharyngeal dynamics in freely moving animals. To do this we are tracking the actions of the pharynx, which is a neuromuscular organ essential for the intake of food. Depending on its diet, the P. pacificus pharyngeal rhythm shifts from a rapid pumping action observed while feeding on bacteria to a lengthier more robust rhythm employed while predatory feeding. Therefore, we are exploring these contrasting dynamics to identify shifts in behaviour associated with feeding mode and diet and additionally identify novel features of predation behaviours during different foraging states.

12. **Bacterial diets are able to modulate life-history treats in C. elegans models of neurodegenerative diseases**

Veuthey, Tania; Burkovski, Andreas

As life expectancy increase worldwide, age-related disorders, such us neurodegenerative diseases (ND), have become more prevalent. Moreover, treatments are only able to attenuate some symptoms, but fail to arrest characteristic neuronal proteotoxicity. Thus, new challenges emerge to science in order to understand molecular basis of these disorders. Lately, the hypothesis that gut microbes affect neurodegenerative diseases through the gut–brain axis is gaining increasing attention and a close relation between the complexity and diversity of gut microorganism and ND has been proposed. The aim of our work was to evaluate the relevance of the microbiota in the progression of proteotoxic-based disorders, assessing the impact of six non-pathogenic bacterial diets on life-history traits in *C. elegans* models of ND, relative to the standard OP50. In a first approach, we found 2 bacteria, *Escherichia coli* K12 and *E. coli* HB101, able to improve locomotion in liquid media, in worm's model of Parkinson disease (PD) at adult day 4, versus *E. coli* OP50. Moreover, an age-dependent locomotion improvement, between larva-L4 and adult day 4, was observed in solid media after feeding PD model´s worms with 4 different bacteria versus *E. coli* OP50. We also observed an increase in the developmental timing of wild-type worms grown in 4 bacteria versus *E. coli* OP50, but more interesting was the accelerated developmental rate selectively found in models of PD and Huntington disease feed with *E. coli* BL21 (DE3). In addition, we observed that using *E. coli* BL21 (DE3) as a food source, L4 larvae of PD models showed a significant increase stress resistance. When the reproductive performance was evaluated, no bacterial diet tested was shown to affect the parameters studied in all worms' models. In order to discard that the observed effect were due to changes in food intake, the pharyngeal pumping was also evaluated, without finding changes using all bacterial diets as a food source. We are currently evaluating aggregate numbers, lifespan and
mitochondrial morphology among others. Our results allowed us to identify bacteria with the ability to drive physiological outcomes and improve health status of *C. elegans* models of neurodegenerative diseases

13. **Behavioral ecology of the worm: cultivating C. elegans in rotting fruit and soil in the lab**

Lee, Jin; Indong, Rocel; Park, Jong-Min; Moon, Je-Hyun

Neurobiology and behavior studies in the worm have revealed the genes and circuitry that regulate C. elegans behaviors in the laboratory. However, the relevance of these neuronal and genetic factors on the survival and reproductive fitness of the worm in its natural habitat is unknown. C. elegans is commonly found in the wild flourishing in rotting fruit and soil. In order to simulate these conditions, we are incubating soil and apples in a plant growth chamber varying temperature, humidity, and day-night cycling, to identify an optimal growth condition for C. elegans. In these optimal conditions, C. elegans can grow in population by more than 3000-fold within 8 days starting from L1 larvae. One of our goals is to characterize the ecological succession of the microbial communities that allow C. elegans to flourish in these habitats. In addition, we find that the C. elegans can migrate to different positions within the

soil-fruit habitat depending upon the environmental conditions. We plan to screen mutants of candidate genes that affect various sensory processes and behaviors in these habitats with the goal of identifying essential factors that contribute to the reproductive fitness as well as the migration of the worm populations. Our overall goal is to establish a method to evaluate the ecological relevance of genes and circuitry that regulate behaviors.

14. Bidirectional Spiraling is a Population-Level Emergent Behavior in C. elegans

Persson, Laura

Many organisms engage in collective behaviors that benefit the survival of the individual. When many individuals simultaneously follow a set of behavioral rules about how to interact with one another and their environment, group behaviors with new functionalities can emerge. This phenomenon of emergent behavior occurs widely in nature, from prokaryotes to honey bees to herd animals. Remarkably, collectives can show complex, high level organization in the absence of any centralized control system, a concept termed “swarm intelligence”. The concept of swarm intelligence has been employed widely in computer science. However, our ability to build biological systems with emergent properties or to perturb biological collectives in predictable ways is limited. Historically, studying these behaviors has been challenging because of the experimental intractability of many of the organisms known for complex group behaviors. Here, we report the discovery of an emergent group behavior in the highly tractable model organism C. elegans. A simple environmental cue in which a lid is added to a previously open agar dish prompts a population of C. elegans to initiate a highly coordinated bidirectional spiral that redistributes the population from the edges of the plate to the center. Notably, we have found that individuals are insensitive to the dish being open or closed, indicating that this behavior is an emergent property of the population. Moreover, individuals can be made sensitive to the cue by the presence of a high density population in the same compartment even when the physical setup precludes their direct interactions. This suggests that the interworm signals that coordinate spiraling behavior in C. elegans are transmitted
“wirelessly”. By perturbing experimental conditions, testing genetic mutants with behavioral defects, and recording neural activity in freely behaving animals, we aim to characterize the etiology of this novel behavior and elucidate principles of emergence in a biological system at high granularity.

15. Building the neuropeptidergic connectome of Caenorhabditis elegans

Ripoll Sánchez, Lidia; R. Schafer, William; Hobert, Oliver; E. Vértes, Petra; Beets, Isabel; Watteyne, Jan; Miller III, David M.; Taylor, Seth R.; Hammarlund, Marc; Weinreb, Alexis; Fernandez, Robert W.; Sun, HaoSheng

The extrasynaptic volume-transmission signaling circuitry modulates and acts in parallel with the synaptically-wired neuronal circuitry to communicate neurons. Indeed, neuromodulatory signaling also forms a complex network, whose structure has not been described for any animal. Since neuromodulation is critical to nervous system function, it is important to map these extrasynaptic signaling networks at a whole-organism level.
To this end, we have used single-neuron gene expression from the CeNGEN database along with diaphanization data for neuropeptide-activated G- protein coupled receptors (GPCRs)

(see De Fruyt et al.) to generate a draft connectome of neuropeptide signaling in C. elegans. We based our network on single-cell neuronal expression patterns of 91 neuropeptide-receptor couples. Network edges were formed when the sending neuron expressed a given neuropeptide, the receiving neuron expressed the cognate receptor, and both neurons projected processes into the same neuropil bundle. We also generated a network with less spatial restriction on edge formation which allowed for potential longer-range signaling between nerve bundles in the same body region. Analysis of neuropeptidergic gene expression suggests that at least some of these mid-range signaling events occur in vivo, particularly between the pharynx and the nerve ring and between the canal-associated nerve and the ventral cord. Since all 302 neurons of the adult hermaphrodite express at least one neuropeptide precursor and nearly all express one neuropeptide GPCR, both the restricted and the midrange networks were extremely dense compared to the synaptic connectome.
The neuropeptide connectome differs greatly in structure from the monoamine and synaptic connectomes, with a very high connection density and a decentralized topology. More than half its neurons participate in a highly interconnected core of very high degree, and several of the most important nodes this core, including PVT, PVQL, PVQR and PVR, are little-studied neurons that may be specialized for peptidergic neuromodulation. Interestingly, some neuropeptide GPCRs show unexpectedly high coexpression with peptide ligands for other receptors, generating signaling cascades that may regulate behavioral states. We expect that the newly-mapped neuropeptide connectome of*C. elegans* will serve as a prototype for other animals and provide new insight into the structure of neuromodulatory networks in bigger brains.

16. C. elegans as a model to characterize the functional impact of a CLTC recurrent mutation underlying intellectual disability and juvenile parkinsonism

Pannone, Luca; Muto, Valentina; Nardecchia, Francesca; Onorato, Giada; Di Rocco, Martina; Barresi, Sabina; Tosato, Federica; Bertuccini, Lucia; Galosi, Serena; Di Schiavi, Elia; Leuzzi, Vincenzo; Tartaglia, Marco; Martinelli, Simone

The CLTC (Clathrin heavy chain polypeptide) gene encodes the widely expressed clathrin heavy chain 1, which is involved in endocytosis, intracellular trafficking, synaptic vesicles regeneration, and neurotransmitters recycling. Recently, we reported that de novo CLTC mutations underlie a wide spectrum of infantile/childhood neurological phenotypes, including developmental and epileptic encephalopathies, movement disorders, and isolated intellectual disability (ID). To our knowledge, no functional studies have been performed so far to dissect the pathogenic consequences of these variants. Here, we characterize functionally the impact of the recurrent c.2669C>T (p.Pro890Leu) change causing ID, global developmental delay, and early-onset parkinsonism using a CRISPR-Cas9-engineered C. elegans strain harboring the aforementioned variant in chc-1, the C. elegans orthologue of human CLTC. Homozygous animals displayed resistance to aldicarb-induced paralysis, indicating defective release of acetylcholine at the neuromuscular junction. In line with this finding, the chc-1 mutants showed a partial depletion of synaptic vesicles at the level of the lateral nerve cords. Dopamine- and GABA-mediated transmission were also affected, as revealed by a partial rescue of the swimming-induced paralysis (SWIP) phenotype observed in a sensitized dat-1 null genetic background and hypersensitivity to pentylenetetrazole (PTZ), respectively. Furthermore, nematodes carrying the mutation had defective synaptic plasticity, as unveiled by learning assays performed following conditioning with high doses of benzaldehyde.

Phenotypic profiling of heterozygous nematodes and of transgenic lines overexpressing the mutant construct pan-neuronally demonstrated a dominant- negative behavior of the pathogenic variant. Automated analysis indicated a slightly defective locomotion of chc-1 mutants. Electron microscope analysis revealed no significant differences in dimension and number of synaptic vesicles in mutant animals. In vitro studies performed on patient's-derived fibroblasts showed a dramatic decrease in transferrin uptake, indicating defective endocytosis. Overall, these findings establish that p.Pro890Leu causes defective intracellular trafficking, which likely results in neurotransmitter depletion in the brain due to aberrant synaptic vesicles formation/turnover.
Keywords: chc-1/CLTC; clathrin; infantile parkinsonism; synaptic vesicles.

17. Characterisation of a Cationic Dopamine-Gated Ion Channel in C. elegans and other invertebrates.

Courtney, Amy; Hardege, Iris; Styfhals, Ruth; Marinkovic, Milena; Jékely, Gáspár; Seuntjens, Eve; R. Schafer, William

Cys-loop ligand-gated ion channels (LGCs) are essential for fast neurotransmission. Vertebrate LGCs include excitatory ACh/5HT3 receptors as well as inhibitory GABA/glycine receptors. However, invertebrate LGCs have diversified significantly, with *C. elegans* having channels activated by novel ligands such as protons, betaine, tyramine, octopamine, histamine and choline as well as channels with "inverted" ion selectivity (excitatory GABA channels and inhibitory ACh channels). However, our understanding of invertebrate LGC function has been restricted to the major model organisms, and the true functional diversity of these receptors is not understood. We addressed this gap by exploring the function of LGCs from a previously neglected invertebrate phyla. We performed phylogenetic analysis on metazoan LGCs and identified LGCs with potentially novel ligands in multiple invertebrate species, including the cephalopod mollusc *Octopus vulgaris* . We performed deorphanization experiments on octopus LGCs using *Xenopus* oocytes and identified the first example of an excitatory dopamine-gated channel in any species. The phylogenetic analysis also revealed that this channel is present in other molluscs, nematodes, annelids, some arthropods and is missing in platyhelminths and vertebrates. Oocyte experiments showed that*C. elegans* also possesses this channel, but unlike the other species which have a homomeric version, worms have a heteromeric version. We now understand the pharmacology and evolutionary history of this channel, but we are also keen to understand its role*in vivo* . There are 12 dopaminergic neurons in *C. elegans* . Combining connectomic data and expression data we identified many neurons which express this dopamine-gated channel and are synaptically connected to dopamine synthesising neurons. It has previously been shown that the release of dopamine upon food encounter leads to a slowing response. We performed optogenetic experiments to control the release of dopamine and observed this slowing response in wild type worms.
We found that dopamine-channel mutants had a faster recovery time back to baseline speed compared to N2, suggesting that this dopamine-gated channel acts to sustain this slowing response. Work is ongoing to understand what specific neurons and circuits are involved in mediating this behaviour.

18. Characterizing the functional impact of genetic variants in neurexin/NRXN1 in C. elegans

Haskell, Dustin; Hart, Michael; Cowen, Mara

Neurodevelopmental and neuropsychiatric disorders are genetically and phenotypically complex and likely often manifest as the result of multiple genetic perturbations and/or disease-associated variants. Neurexins, a family of synaptic adhesion proteins plays critical roles in neuron and behavioral development, and variants in *NRXN1* have been strongly associated with autism spectrum, schizophrenia, and Tourette's syndrome. To better understand the mechanistic and behavioral roles of neurexin in health and disease, we are using*C. elegans* to understand the functional impact of disease-associated variations within the *NRXN1* gene. *NRXN1* consists of three major isoforms (alpha, beta, and gamma), and alternative splicing of within these major forms can generate hundreds of unique proteins. We set out to characterize 10 isoforms of human*NRXN1* identified from control (5 control isoforms) or schizophrenia proband hiPSCs, which harbor a 3' *NRXN1* deletion (5 mutant isoforms). We generated *C. elegans* that express each human *NRXN1* isoform in all neurons in (*nrx-1(wy778)*) mutants, which lack the singular *C. elegans* neurexin ortholog (*nrx-1*). Additionally, we characterized the impact of a conserved missense mutation identified in an autism proband (L18Q) that was mutated using CRISPR/Cas9 in the *nrx-1* gene (L16Q), and expressed *nrx-1* in all neurons with and without this mutation. For each *NRXN1* isoform and the conserved *nrx-1* variant, we assayed protein expression, localization, neuron morphology, and impact on behavior. We find that variants in*C. elegans* NRX-1 alter multiple foraging related behaviors. Similarly, the disease-specific isoforms in human *NRXN1* show altered expression/localization in neurons, mild morphological defects, and behavioral phenotypes compared to control isoforms in *C. elegans* . Ongoing work includes in-depth characterization of
these *NRXN1* variants to fully understand the mechanistic role neurexin plays in neurodevelopmental changes, however this work provides a convenient and tractable model to screen for functional conservation of genes and disease-associated variants in a reasonably high-throughput fashion.

19. Circuit and Molecular Mechanisms of an Associative Learning Task

Colinas Fischer, Susana; Molina-Garcia, Laura; Clark, Emma; Lin, Lucy; Barrios, Arantza

The ability of neural circuits to be changed by experience, optimising an organism's behaviour, is key to survival. We are dissecting the role of the neuropeptide PDF in mediating aversive olfactory learning to benzaldehyde in C. elegans. When benzaldehyde is paired with starvation, an aversive experience, C. elegans' response to benzaldehyde switches from attraction to repulsion (Lee 2010, Lin 2010). Here we show that both PDF-1 and PDF-2 mediate this form of aversive learning and seek to describe the underlying circuit.
Benzaldehyde is sensed primarily by the AWC neuron, which synapses onto first-level interneurons AIB, AIY and AIA to regulate naïve chemotaxis (Bargmann 1993, Chalasani 2007). The switch from attraction to repulsion during aversive learning has been shown to be driven by changes at the AWC- AIB synapse (Cho 2016). We find that AWC-ablated animals have a dampened response to BZ, but we still observe a switch in response from attraction to repulsion after aversive conditioning, indicating that learning can occur elsewhere in the circuit. Given that we also know that PDF is required to mediate aversive learning, we are looking to find which neurons are the relevant source of and target for PDF signalling in this behaviour. To find the source, we are using an intersectional Cre-Lox strategy to return

physiological levels of PDF-1 to specific neurons. To find the target neurons we are also using an approach that exploits the strength of the Cre-Lox system, to restore PDFR-1 function to certain neurons selectively. We are using calcium imaging to record the activity of the first line of interneurons (AIA, AIB and AIY), and, once identified, we will also image the neurons we implicate in the PDF circuit for aversive learning, to see how the information flow through the circuit changes with learning.
Given that associative learning is a highly conserved behaviour, understanding how this simple circuit is capable of supporting aversive learning will provide us with principles that can be applied to further understand how learning occurs in more complex nervous systems.

20. Clarinet (CLA-1) recruits RIMB-1/RIM-binding protein and UNC-13 to orchestrate neurotransmitter release.

Krout, Mia; Richmond, Janet

Synaptic transmission requires the coordinated activity of multiple synaptic proteins that are localized at the active zone (AZ). As in other organisms, *C. elegans* AZs contain a cytomatrix of conserved multi-domain proteins, including: SYD-2/Liprin-a, UNC-10/Rab3-Interacting Molecule (RIM), RIMB- 1/RIM-Binding Protein (RBP), ELKS-1/ELKS, UNC-13/Munc13, and UNC-2/P/Q-type calcium channels. Functional interactions between these proteins orchestrate the docking and priming of vesicles, calcium-channel localization and function, and regulate the probability and timing of neurotransmitter release. Other AZ proteins appear to be more species specific including; Mammalian Piccolo and Bassoon, and fly BruchPilot and Fife, but even these proteins exhibit domain conservation and appear to have overlapping functions with the conserved AZ components. We previously identified a novel*C. elegans* protein named Clarinet (CLA-1) based on homology to the AZ proteins Piccolo, RIM/UNC-10 and Fife. The *cla-1* gene encodes three isoform pairs grouped by size: long, medium and short, all of which appear to be pan-neuronally expressed. All CLA-1 isoforms contain C-terminal PDZ and C2 domains, in common with Piccolo, Fife and RIM, which tether CLA-1 at the AZ. At the neuromuscular junction (NMJ) *cla-1 null* mutants exhibit reduced release and pronounced synaptic depression, which is exacerbated in *unc-10* mutants (Xuan et al., 2017). The mechanisms underlying the role of CLA-1 remain unresolved, but the data outlined above implicate CLA-1 in the regulation of exocytosis, in coordination with other components of the AZ cytomatrix including UNC-10. To gain mechanistic insights into the role of CLA-1, in collaboration with the labs of Peri Kurshan and Hongkyun Kim, we examined the relative contributions of different CLA-1 isoforms to the function and organization of the AZ and explored the relationship between CLA-1 and UNC-10. Our data show that CLA-1 regulates calcium channel levels at the synapse via the stabilization of Rim-binding protein, RIMB-1. In addition, CLA-1 exerts a RIMB-1-independent role in the localization of the priming factor UNC-13. We propose that CLA-1 and UNC-10 act partially redundantly to organize the synaptic machinery through the differential regulation of multiple AZ proteins to support release.

21. Constructing a tool box for imaging and stimulating pharyngeal neurons to understand foraging behavior in C. elegans

Liu, Jun; Bonnard, Elsa; Alvarez, Luis; Scholz, Monika

In C. elegans, the circuit controlling feeding comprises only 20 neurons and is separate from the 282 somatic neurons, yet it controls food intake and modulates feeding rate effectively. The small pharyngeal circuit is ideally suited to understand the function of a contained, nearly

isolated circuit in an intact, behaving animal. Specifically, we want to understand: I) What is the function of individual pharyngeal neurons during foraging? II) How do pharyngeal neurons communicate with the extra-pharyngeal neurons to coordinate foraging behavior? III) What is the activity of individual pharyngeal neurons during foraging? To these ends, we aim to create a toolbox that targets individual and subsets of pharyngeal neurons for expressing a range of optogenetic tools and the genetically-encoded calcium indicator GCaMP.
We will use the cGAL and split cGAL system developed by the Sternberg Lab which is adapted from the GAL4-UAS system for the C. elegans community. We create driver strains by selecting a single promoter (cGAL) or two intersecting promoters (split cGAL), to achieve specific expression in targeted neuron(s). We then cross these drivers with their UAS effector strains, such as GFP, optogenetic activator/inhibitor and GCaMP, to achieve neuron- specific expression of fluorophores or other desired proteins.
To identify promoters that drive unique expression in pharyngeal neurons, we have curated information from the literature and transcription database (CenGen). We will generate different "promoter::cGAL" driver lines and then verify the expression using the GFP effector lines and the neuroPAL multicolor Atlas. Successful candidates will then be crossed to other optogenetic and GCaMP effector lines for targeted neuronal manipulations and calcium imaging of foraging animals.
We have obtained some of the transgenic driver lines and tested them with GFP effector. We will present our progress in creating such a toolbox. We expect this strain collection to be a valuable tool to understanding the connection between the activity of all neurons in a small circuit and feeding behavior

22. Context-dependent reversal of odorant preference is driven by inversion of the response in a single sensory neuron type

Pandey, Anjali; Sengupta, Piali; Khan, Munzareen

Animals must respond to their environment in a robust but flexible manner in order to adapt to constantly changing stimuli. Along with sensory cues, animals integrate their past experiences, internal state, and environmental context to make the most optimal behavioral decisions. Olfaction is a critical sensory modality which animals use for social communication, food-search behaviors, and to avoid predators. Multiple sensory cues are integrated in a context-dependent manner to modulate olfactory behavior. How context-dependent olfactory behavior is regulated at the cellular and molecular level is not fully understood.
We find that while C. elegans is normally attracted to a point source of the odorant, 1-hexanol (HEX), animals are instead repulsed by HEX when a different attractive odorant such as isoamyl alcohol (IAA) is ubiquitously present and saturating during the behavioral assay. The dichotomy of these behavioral responses is mediated by two distinct signaling pathways. Attraction to HEX requires the AWC neurons, whereas avoidance of HEX under IAA saturation conditions can be mediated by either the AWC or ASH neurons. As described previously, the AWC neurons are inhibited by HEX ("odor OFF" response). Surprisingly, under IAA saturation conditions, addition of HEX instead elicits a robust increase in intracellular calcium levels in AWC neurons ("odor ON" response). These novel "odor ON" responses in AWC to HEX under IAA saturation conditions requires the Gα protein ODR-3, whereas the HEX alone OFF response is mediated by other Gα proteins and the ODR-1 receptor guanylyl cyclase. To identify additional molecules involved in the context-dependent "odor ON" response in AWC, we performed a forward genetic screen. We have isolated several mutants that are attracted

to HEX even under IAA saturation conditions including likely additional alleles of odr-3. The identities of these genes, mutations in which alter the HEX responses in AWC, will provide a more complete understanding of the molecular mechanisms underlying context-dependent olfactory plasticity in a single sensory neuron type.

23. Control of synapse formation by novel extracellular interactions

Mialon, Morgane; Patrash, Liubov; Bessereau, Jean-Louis; Pinan-Lucarre, Berangere

The diversity and specificity of synapses rely upon core organizing Cell Adhesion Molecules (CAM) that regulate contact initiation, synapse formation, maturation, maintenance and functional plasticity. We recently identified that the ACR-16 acetylcholine receptor, well characterized at neuromuscular junctions, is also present at neuron-to-neuron synapses along the ventral cord. Using a fluorescent reporter of the ACR-16 acetylcholine receptor, we performed a visual screen upon random mutagenesis to identify mutants with altered ACR-16 containing neuron-to-neuron synapses in the ventral nerve cord. One mutant caught our attention because the ACR-16 acetylcholine receptor was no longer synaptic and appeared diffuse at the neuronal surface. This phenotype was consistent with a mutation in a core synaptic organizer. We identified the mutated gene, which encodes a member of the Immunoglobulin superfamily CAM. This CAM shows a strikingly specific localization at ACR-16 neuro-to-neuron synapses. Moreover, we found that a known in vitro binding partner of this CAM is also very specific of ACR-16 neuron-to-neuron synapses. Overall, our data suggest that we identified two novel synaptic molecules that might form a bridge across neurons and control synapse formation and/or maintenance in C. elegans. Interestingly, orthologs of this CAM are associated with a wide spectrum of human neurodevelopmental and neuropsychiatric disorders, and might control synaptogenesis in mammals.

24. Detecting signatures of evidence accumulation in the neurogenic control of pharyngeal pumping

Alvarez, Luis; Liu, Jun; Scholz, Monika; Hillman, Elizabeth M.C.; Campos, Citlali Perez; Yan, Richard Wenwei

During motor tasks, animals continually integrate sensory information about the environment to make informed decisions. The nematode Caenorhabditis elegans acquires food by the pumping action of its pharynx: a neuromuscular organ that is controlled by a subcircuit comprising 20 neurons. Worms adapt their pumping rate to the available food in the environment. It has been suggested that worms sample their surroundings by pumping. Based on their food sampling, worms adjust their pumping rate, possibly using a decision process. We are interested in identifying the neurons, signals, and transfer functions in the worm that enable this decision-making process. To study the activity of all pharyngeal neurons, we generated C. elegans strains that express GCaMP6 and TagRFP exclusively in pharyngeal neurons, thus enabling uniquevocal neuron identification. We record worms exposed to different levels of food using a combination of microfluidics and SCAPE microscopy. SCAPE is a novel light-sheet microscopy method that allows for whole-brain imaging in 3D at high volume rates with cellular resolution and low phototoxicity. We will show our advances in using these techniques to image neural activity in C. elegans while feeding.

25. **Discovery and characterization of a novel suppressor of SOD1 ALS-associated neurodegeneration**

Lin-Moore, Alexander; Hart, Anne

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease with no known cure or effective therapies. While genetic analyses of patients have found causal mutations in several key genes, no unifying cellular mechanisms have been identified that explain ALS-associated neurodegeneration. The Hart lab has previously generated a knock-in model of SOD1 G85R, a causative mutation in heritable ALS, and has leveraged this model to perform the first unbiased screen for suppressors of neurodegeneration in an in vivo ALS model. We have identified a candidate gene whose mutation leads to significant rescue of glutamatergic and cholinergic neurodegeneration associated with SOD1 ALS. Characterization of these genes, including cell autonomy, conservation with mammalian orthologues, and discovery of physical and genetic interactors, is ongoing. Our goals for this study include the identification of novel cellular pathways of ALS-associated neurodegeneration, the linking of mechanisms between disparate ALS models, and the identification of candidate therapeutic targets whose knockdown may ameliorate or suppress neuron death in ALS.

26. **Dissecting signaling effector functions: How does IL-17 modulate neuronal circuits?**

Ramirez, Nelson; de Bono, Mario

The interleukin 17 (IL-17) cytokine homolog ILC17.1 regulates a range of phenotypes in C. elegans, including O2 escape behavior, associative learning, immunity and longevity. Two conserved proteins, the paracaspase MALT-1 and the IKBzeta homolog NFKI-1, are effectors of the neuromodulatory signaling cascade triggered by ILC17.1. Precisely how MALT-1 and NFKI-1 signaling alter neuronal responses remains, however, largely unknown. We aim to understand how these two proteins change neuronal properties to influence behavior. Preliminary data suggest MALT-1 and NFKI-1 regulate the levels of specific mRNAs in specific neurons of the circuit. MALT-1 most likely affects stability of particular mRNAs while NFKI-1 associates with transcription factors and chromatin remodelers.
We are using RNAseq to profile specific cells of the oxygen sensing circuit, and to identify genes that are differentially expressed in malt-1, nfki-1 and ilc-
17.1 mutants. By comparing expression profiles across all three mutants we expect to highlight commonly regulated genes. To validate these candidates we will analyze cellular and behavioral phenotypes and test genetic and physical interactions with known components of the cascade. We expect to gain mechanistic insight into how MALT-1 and NFKI-1 modulate neuronal responses.
Roles for MALT-1 and NFKI-1 independent of mRNA regulation are also possible. To explore this possibility, we are using TurboID, a proximity labeling technique that highlights protein interactors in vivo, including transient ones. We will test mutants of candidates we identify using behavioral assays and calcium imaging. A similar, non-additive signature would be expected for candidate interactors when compared to mutants of other IL-17 pathway components.
In mammals altered IL-17 signalling has been associated with autism and defects in cortical development. We hope to use C. elegans to identify conserved mechanisms potentially relevant to these mammalian phenotypes.

27. Elucidating the roles of extracellular signals in the sexual behavior of Caenorhabditis afra

Shapira, Shachaf; **Wolfson, Eya**

Species in the Elegans supergroup of the subgenus Caenorhabditis can be distinguished by two very different mating systems: androdioecy, which consists of males and hermaphrodites, and dioecy, which consist of males and females. The ways in which significant changes in the sexual composition of a species affect the behavioral patterns of its individuals, and the underlying sensory cues and neurological outputs have remained mostly unexplored. Strikingly, we found that, while in natural populations of C. elegans hermaphrodites remain largely passive or even attempt to escape male mating endeavors, females of the gonochoristic C. afra species exhibit great interest in males and actively participate in the mating process, often acting as its initiators.

In order to examine whether the observed response of C. afra females to males depends on secreted chemical cues, we tested their attraction to male- derived conditioned media (MCM), and found C. afra females to be significantly and specifically attracted to it.

Since it is known that C. elegans ciliated sensory neurons release extracellular vesicles (EVs) carrying transmembrane proteins that mediate mating- related behaviors (Wang Juan, et al., 2014), we investigated whether the observed MCM chemoattraction is due to the release of EVs by C. afra males. We found that C. afra MCM share some properties similar to those of EVs.

Moreover, we have developed a new method for isolating the vesicles from the conditioned media, which allows us to characterize them and further explore their effect on C. afra sexual behavior.

Our results highlight some of the less-known discrepancies in female and hermaphrodite mating behavior, and provide a glimpse into some of the mechanisms at play.

28. Establishing function for ST7, a conserved family of polytopic membrane proteins

Schoen, Hanna; Flynn, Sean; Lashmanova, Ekaterina; Amin-Wetzel, Niko; Barratt, Stephen; de Bono, Mario

The highly conserved gene F11A10.5 is the C. elegans ortholog of human Suppressor of tumorigenicity 7 (ST7) and Suppressor of tumorigenicity 7-like (ST7L). ST7 genes can be found in genomes of most metazoa but is absent in the genomes of single-celled eukaryotes. Despite the name, the biological function of ST7 proteins is unknown. ST7 are membrane proteins, consisting of three transmembrane (TM) domains and a large, putatively cytoplasmic domain between TM domains 2 and 3. Initially studies of human tumour-derived cell lines suggested that ST7 is a tumour suppressor gene (Zenklusen et al., Nat Genet., 2001) however subsequent studies have cast doubt on this (Dong and Sidransky, Clin Cancer Res., 2002). In mouse and humans, ST7 and ST7L proteins are expressed broadly, including in the nervous system.

A forward genetic screen for mutants defective in C. elegans aggregation behavior identified seven alleles of F11A10.5, including multiple nonsense alleles. A battery of behavioral tests indicate that F11A10.5 mutants, whilst otherwise healthy, have the signature behavioral phenotypes of a disrupted oxygen hub-and-spoke circuit namely: defects in escape from 21% O2, elevated escape from CO2 and robust escape from hypoxia.We tagged endogenous

F11A10.5 with mNeonGreen using CRISPR-Cas9. Fluorescence microscopy reveals a predominantly neuronal expression pattern, with most neurons showing fluorescence. To gain insights into where F11A10.5 functions we knocked in a construct encoding GFP and an auxin-inducible degron. The knockin animals were wild type for escape from 21% O2 and showed the expected neuronal expression pattern. Introducing pan-neuronally expressed TIR1 into the F11A10.5::GFP::AID background did not confer any obvious phenotype in the absence of auxin. Adding auxin to this strain recapitulated the F11A10.5 mutant phenotype. I am now expressing TIR1 in individual and sets of neurons to identify where F11A10.5 functions to promote oxygen sensing behaviors.
Collectively, our data shows that F11A10.5 shapes the oxygen sensing neuronal circuit activity to regulate behavior. We have an opportunity to identify the function of this family of proteins in the biology of healthy and diseased individuals.

29. Expressing human epithelial Na channel subunits in C. elegans to model human salt taste

van Vuuren, Laura; Jansen, Gert; Hoorn, Ewout

Human salt taste is one of the main drivers of dietary salt intake and directly correlates with blood pressure. Salt sensitivity and preference vary among people, but the underlying molecular mechanism of this variation is unknown. Many studies have shown that the epithelial sodium channel (ENaC) is the main salt sensor involved in salt taste in rodents and humans. In the kidney, the activity of ENaC is regulated by proteases. Recently it was shown that differences in the salivary proteome correlate with salt sensitivity. We hypothesize that salivary proteases regulate ENaC open-probability and salt taste.
We use C. elegans as a model to study human salt taste. C. elegans is attracted to NaCl concentrations up to 200 mM and avoid higher NaCl concentrations. Low NaCl concentrations are mainly sensed by the ASE neurons and high concentrations by the ASH nociceptive neurons. Thus far, there are no indications that ENaC channels play a role. We will generate a humanized NaCl-taste worm model that expresses all three human ENaC subunits in the ASH cells. We use a two-step approach using CRISPR/Cas9 induced homology directed repair for each subunit. We first introduced a 3.7 kb sra-6::gfp construct in a save harbor locus on chromosome I. This strain showed proper GFP expression in the ASH neurons. Subsequently, we introduced the SCNN1A gene, encoding the ENaCα subunit, after the sra-6 promoter and fused in frame with GFP. In these animals we see weak GFP expression in the cell bodies of the ASH neurons. We used the same approach to express SCNN1B, SCNN1G and SCNN1D genes fused with mScarlet, tagBFP2 and GFP, respectively. We expect that the ENaC expressing animals will be less attracted or even repelled by salt. To reduce interference of the C.

30. Expression of trx-1 correlates with intrinsic regenerative capacity

Grooms, Noa; Fitzgerald, Michael; Ureña, Samuel; Schulting, Leilani; Chung, Samuel; Grooms, Noa

A conditioning lesion of the peripheral sensory axon triggers robust central axon regeneration in mammals, suggesting that lesion conditioning could drive powerful therapies for neuroinjuries. Despite being studied for more than 30 years, lesion conditioned regeneration

remains poorly understood, and progress is limited by low throughput in vertebrate models. To expedite research in the field, we developed a model for lesion-conditioned regeneration in *C. elegans* . Our model employs green fluorescent protein (GFP) to cell-specifically label the ASJ neuron under a*trx-1* promoter. Under our model, axotomy alone does not trigger regeneration; however, we condition the ASJ to regenerate by concomitantly cutting its dendrite. We previously demonstrated that mutations to genes in the sensory pathway can chronically condition and increase regenerative capacity without a conditioning lesion. During prior studies, we noticed by eye that ASJ conditioning by mutation or physical lesion also leads to increased ASJ fluorescence. This increase in brightness suggests that ASJ fluorescence could be a reporter of regenerative capacity, which is supported by mammalian TRX being a regeneration-associated gene, which are upregulated following conditioning. In this study, we characterize the upregulation of*trx-1* under conditioning interventions via GFP expression and examine the role of*trx-1* in conditioned regeneration.
We quantified changes in ASJ fluorescence by imaging mutant and postsurgery animals along with calibrated fluorescent beads. ASJ fluorescence significantly increases in conditioned mutants and dendrite-cut animals. We demonstrate that *trx-1* mediates conditioned regeneration but inhibits non-conditioned regeneration. Finally, we tested the utility of our model by using a fluorescence-based proxy to screen for genes underlying the conditioned pathway. We used ethyl methanesulfonate to stochastically introduce mutations into a conditioned strain and selected for offspring with decreased ASJ fluorescence. We isolated twelve strains, and six show significantly reduced frequency of ectopic axon outgrowth, a form of conditioned regeneration without lesion. A reduction in ectopic outgrowths suggests a disruption in the conditioning pathway. In summary, we demonstrate a correlation between *trx-1* expression and conditioning-driven intrinsic regenerative potential that can be leveraged to efficiently evaluate regenerative capacity.

31. Fate of a Cleavage Product of APL-1, the C. elegans orthologue of Human APP

Li, Chris; Yang, Ji-Sup; Mercado, Alessandro; Charles, Saidra; Zavalunova, Jessica

Alzheimer's disease is a neurodegenerative disease that affects over 5 million Americans. The disease is characterized cellularly by the presence of neurofibrillary tangles, which is composed of hyperphosphorylated tau, and senile plaques, whose major component is the beta-amyloid peptide. The beta-amyloid peptide is a cleavage product of the amyloid precursor protein (APP); mammals have a family of APP proteins, which includes APP, APLP1, and APLP2. The *APP* gene family is essential for viability, making it difficult to study its function because of the overlapping functions among family members. *Caenorhabditis elegans* contains only one APP gene, *apl-1* . Like other family members, APL-1 is a single pass transmembrane protein, which is cleaved to release a large extracellular and smaller intracellular fragment. Knockouts of*apl-1* cause larval lethality; however, when a transgene encoding full-length APL-1 or only the extracellular fragment of APL-1 is introduced, the lethality phenotype is rescued. This rescue raises the question of where the cleaved extracellular fragment migrates and binds to activate a pathway for viability. After much trial and error, we devised a transgene in which the extracellular and intracellular domains are tagged with sfGFP and wrmScarlet, respectively; this transgene is functional as it rescues the *apl-1* null allele. Our goal is to identify cells to which the extracellular fragment binds.

32. FLWR-1 facilitates synaptic recovery after strong optogenetic stimulation of the neuromuscular junction

Seidenthal, Marius

The Flower protein is involved in synaptic vesicle (SV) recycling during strong synaptic stimulation of Drosophila neuromuscular junctions (NMJs) and cultured hippocampal rat neurons (Yao et al., 2017). This highly conserved protein was shown to be an integral part of SVs and is proposed to mediate synaptic Ca2+ influx upon intense SV fusion, thus facilitating SV recycling. While the Flower protein has been investigated in Drosophila and (in part) in mammalian cells, no study has yet explored its role in SV recycling at the C. elegans NMJ and examined its functional relation to other Ca2+-channels which were implicated in SV recycling. We show that FLWR-1, the C. elegans homolog of Flower, is expressed in several neuronal cell types and colocalizes with a synaptic active zone marker of cholinergic motor neurons. Using a knockout mutant of FLWR-1 we could show that while basal locomotion is barely reduced, recovery of swimming cycles after intense optogenetic stimulation of motor neurons is slowed. This defect could be rescued by expression of FLWR-1 under its endogenous promotor. As FLWR-1 is also expressed in muscle, we further explored its neuronal function by electrophysiology. While evoked postsynaptic currents (ePSCs) at the flwr-1 NMJ displayed no difference to wildtype (WT) without or with low optogenetic stimulation of motor neurons. However, upon prolonged strong stimulation, flwr-1 mutants exhibited a faster decline of ePSCs, i.e. increased synaptic fatigue.
Next, we will investigate the role of FLWR-1 in SV recycling. For this we will use a pHluorin-based assay (pOpsicle, abstract by Seidenthal et al.) to compare the SV recycling rates in mutant and WT after strong stimulation. Further, the interaction of FLWR-1 with other proteins involved in Ca2+- conductance and -binding will be tested. Last, by electron microscopy combined with optogenetic stimulation and high-pressure freezing, we will visualize potentially defective SV trafficking and recycling in flwr-1 mutants.
Yao et al. (2017). *PLoS Biol, 15* , e2000931. PMID:28414717

33. From symmetric building blocks to neural synchronization in the connectome

Avila, Bryant; **Augusto, Pedro**; Makse, Hernan; Zimmer, Manuel

Across species, large scale activity recordings showed that neurons coordinate their activity forming globally correlated population states. The underlying circuit mechanisms that give rise to such dynamics are unknown. We propose, that the neuronal connectome must be optimally wired to support such dynamics. In our previous work, performing whole brain Ca2+recordings, we showed that motor programs are represented by globally coordinated brain dynamics with large groups of synchronized neuronal ensembles. Moreover, we recently showed that coordination between these large groups of neurons depend upon high order features of the connectome such as input symmetries and rich club architecture (See abstract by Uzel et al.). Here, by combining new graph theoretical concepts and experimental testing, we aim to unveil how building blocks of the connectome give rise to a functional neuronal network.
Synchronicity is a fundamental notion of neuronal circuits and it is essential to their function. Our previous studies (Morone and Makse,
2019) suggested that network synchronicities depend on localized symmetries in neuronal wiring. Several studies report on such symmetries in the C. elegans connectome. Motor

neurons present in the ventral cord are organized in repeating units (Haspel and Donovan, 2011) and symmetry groups (Morone and Makse, 2019). Such symmetry features can be identified as a collection of neurons that when swapped preserve the connectivity matrix of the network.
Our hypothesis is that symmetries are important for synchronizing neuronal population activity. To test this hypothesis, we compare the symmetries of the connectome with nervous system wide activity recordings. Specifically, we experimentally record population activity in the ventral cord motor system to establish functional connectivity matrices. To assure single cell resolution recordings and accurate neuronal identification, we immobilize animals in a microfluidic chip and record pan-neuronal nuclear GCaMP6f together with the NeuroPALmulticolor neuronal atlas.
Our data not only confirms that symmetries describe the correlated neuronal activities corresponding to the forward-/reverse- crawling brain states, but also uncover undescribed nested smaller groups of synchronized neurons. To experimentally scrutinize our hypothesis, we will apply laser ablation targeting specific neurons that specifically disrupt the theorized symmetry groups, therefore expecting to disrupt the synchronicity.

34. Functional and behavioural insights into properties of acid-sensing DEG/ENaCs

Kaulich, Eva; Carroll, Trae; Ackley, Brian D.; Walker, Denise S; R. Schafer, William; Tang, Yi-Quan; Hardege, Iris; Nehrke, Keith

Acid-sensing ion channels (ASICs) are members of the diverse family of degenerin/epithelial sodium channels (DEG/ENaCs). They are important for a wide range of physiological functions in healthy organisms, such as gut function and synaptic transmission, however, they play important roles in disease, as acidosis is a hallmark of inflammation. To understand their function, we performed an electrophysiological screen for acid-sensitivity on all 30 subunits of the *C. elegans* DEG/ENaC family in *Xenopus* oocytes. We identified four new members that were acid-inhibited (ACD-5, DEL-4, DELM-1 and UNC-105 in addition to the previously described ACD-1) and for the first time we identified three acid-sensitive DEG/ENaCs that were activated by acidic pH (ASIC-1, ACD-2 and DEL-9), making them functionally similar to the vertebrate ASICs. We also observed modulatory diversity by the trace element zinc and to the anti-hypertensive drug amiloride. Acid-sensitive DEG/ENaCs were found to be expressed in both neurons and non-neuronal tissue, highlighting the likely functional diversity of these channels. We focussed on a subset of acid-inhibited DEG/ENaCs and their regulatory role in the defecation motor program (DMP), an ultradian clock, and found that they control proton homeostasis and calcium transient which translates in aberrant motor behaviour. Our findings provide a framework to exploit the *C. elegans* channels as models to study the function of these acid-sensing channels *in vivo,* as well as to study them as potential targets for anti-helminthic drugs.

35. Gap junction-dependent electrical coupling in muscular organs in C. elegans, analyzed by voltage imaging

Wirt, Christin; Bergs, Amelie; Gottschalk, Alexander

Gap junctions (GJs) function as clustered intercellular channels between neighboring cells, in muscular organs and between neuron, allowing ions, molecules and metabolites, but also

electrical impulses to pass the channels. The molecular composition of GJs affects functional properties like open- probability, rectification etc., which subsequently determine how cellular networks can be electrically compartmentalized. Previous studies have shown that GJs in C. elegans are essential for the electrical coupling between cells, and that mutations of the subunits (innexins) cause, for example, impaired locomotion (Liu, 2013). To contribute to the understanding of GJ properties and how innexins mediate compartmentalized cellular networks in the pharynx and body wall muscles (BWMs), genetically encoded voltage indicator (GEVI) imaging was used. As suggested from earlier electrophysiological studies, voltage imaging showed that repolarization in the pharynx occurred in a spatiotemporally compartmentalized manner (Azimi Hashemi, 2019). We compared the wild type to inx-6 and inx-7 mutants, which encode innexins at the 'border' of the compartmentalization domain (metacorpus- isthmus). Using the first derivative of the voltage signal, 'optoEPGs' of serotonin-stimulated pharyngeal pumping could be obtained. optoEPGs, unlike classical EPGs, also provide spatially resolved information. We found that the average delay of repolarization from anterior to posterior pharynx differed significantly among the mutants. We also investigated to which extent gap junctions affect coordination in neighboring BWM cells. For this, spontaneous activity of muscle ensembles was recorded in wild type animals and compared to unc-9, inx-11 and inx-16 mutants. Adjacent muscle cells showed highly coordinated, simultaneous activity in the wild type, while unc-9 mutants revealed the highest degree of uncoordination. Significant differences were also found in the coordination of muscle cell pairs within muscle ensembles of inx-16 mutants, as well as in the coordination of whole muscle ensembles between inx-16 mutants and wild type. inx-11 and inx-16 mutants did not exhibit the same degree of unsynchronized activity as unc- 9 mutants did. In the long term, we aim to address various gap junction ensembles across the muscular and nervous systems.
Azimi Hashemi. PMID: 31371514Liu. PMID 24130800

36. Genetic Analysis of Mate Recognition in C. elegans Males

CHEN, TSE-YU

Mating is a conserved social behavior that transfers genetic variations to maintain phenotypic diversity for environmental adaptation. To precisely and efficiently locate conspecific and reproductive mates, animals utilize multiple sensations to decode the information of species, sexes, and reproductive status. C. elegans males recognize suitable mates partly through physical contact, whereas the genetic basis of contact-mediated mate recognition in males is not fully understood. Here, we find that osm-5, che-2, and klp-6 mutant males do not recognize hermaphrodites in the context of mate searching behaviors, suggesting that these genes are essential for contact-mediated mate recognition. Functional mapping of these genes suggests that they likely function in both ray type A and type B sensory neurons. Our study thus provides a genetic framework to investigate the neural mechanisms of contact-mediated mate recognition in C. elegans males.

37. How does C. elegans recognize the bacterial odors of its microbiome?

Glater, Elizabeth; Chai, Victor; Church, Emily; Taylor, Charles

Chemosensation plays a central role in driving behavioral outcomes, including selecting food sources, finding mates, and avoiding threats and predators. How the nervous system

discriminates among natural chemosensory stimuli, which are often comprised of complex blends of molecules, is not well understood. The nematode C. elegans is an excellent model organism to address this question. While much is known about the neurons and signaling pathways involved in responses of C. elegans to individual chemicals (reviewed in Ferkey et al., 2021), the mechanisms underlying detection of complex natural stimuli remain poorly understood. We are investigating how C. elegans discriminates among different odor blends released by bacteria, their major food source. Specifically, we are examining CeMbio, a simplified microbiome consisting of 12 species that represents the bacteria in C. elegans natural environment (Dirksen et al., 2020). We have found that C. elegans shows a strong preference for bacterial patches of Pantoea nemavictus BIGb0393 over other CeMbio bacteria. We hypothesized that this bacterium releases specific volatile odorants that attract C. elegans.

Consistent with our hypothesis, we found that this bacterium releases high amounts of isoamyl alcohol, a previously described volatile attractant for C. elegans (Bargmann et al., 1993). Moreover, BIGb0393 is hypothesized to be a nutritious food source for C. elegans because it supports relatively fast development time from L1 to adult compared to other CeMbio species and is a relatively weak colonizer of the intestine (Dirksen et al., 2020). Our goal is to define the chemical signatures of the C. elegans microbiome and the neuronal circuitry involved in discriminating among these naturally occurring odor mixtures.

References:

Bargmann CI, Hartwieg E, Horvitz HR (1993) Odorant-selective genes and neurons mediate olfaction in C. elegans. Cell 74:515–527.

Dirksen P, Assié A, Zimmermann J, Zhang F, Tietje A-M, Marsh SA, Félix M-A, Shapira M, Kaleta C, Schulenburg H, Samuel BS (2020) CeMbio - The Caenorhabditis elegans Microbiome Resource. G3 Genes|Genomes|Genetics 10:3025–3039.

Ferkey DM, Sengupta P, L'Etoile ND (2021) Chemosensory signal transduction in Caenorhabditis elegans Iino Y, ed. Genetics:iyab004.

38. Identifying factors that influence muscle atrophy of C. elegans in space microgravity

Kim, Ban Seok

Deep space travel is on the horizon. However, long-term microgravity exposure has several effects on the human body including muscle atrophy. The cause of this muscle atrophy at the cellular level is unknown. Previous gene and protein expression studies with C. elegans in space have shown decreased expression of muscle development genes. Here, preliminary data of space flown worms on the International Space Station showed a 28.3% decreased body wall muscle cell size compared to ground control samples when normalized to body size. To confirm the effect of microgravity on C. elegans muscle, we cultivated worms in a 3D-clinostat that simulates microgravity conditions by constantly rotating the worms in 2 different axes dispersing the gravity in all directions. Preliminary results show that simulated microgravity also decreases body wall muscle cell size. We wondered what genetic factors may be causing muscle atrophy in C. elegans. To this end, we investigated another form of muscle atrophy by starving L4 worms for 30 hours. This level of malnutrition decreased muscle cell size by 26.4% when normalized to body size. Among several mutants tested we identified clp-4, which encodes a calpain family protein predicted to be involved in proteolysis, that suppressed muscle atrophy to only 17.8%. We are continuing to identify other factors that control muscle

atrophy in C. elegans and plan to test whether they are also involved in microgravity-dependent muscle atrophy.

39. Identifying novel interactors of the guanylate cyclase GCY-22 involved in NaCl chemotaxis

Jansen, Gert; Rademakers, Suzanne

C. elegans senses salts in its environment using the ASE neurons. Detection of salts occurs in sensory organelles, called primary cilia. cGMP signalling plays an important role in salt detection. The receptor-type guanylate cyclase (rGC) GCY-22 is involved in the response to NaCl in the environment. We generated a full-length GFP knock-in in the gcy-22 gene. GCY-22::GFP shows unique localization to the ciliary tip and periciliary membrane compartment (PCMC) of one ciliated neuron, ASER. Our goal is to understand the molecular mechanisms that regulate its trafficking and unique localization.
To identify proteins that physically interact with GCY-22, we performed mass spectrometry after immunoprecipitation to identify proteins bound to GFP-tagged GCY-22. Next, we study where the identified candidate interacting proteins are expressed and localized. Mutants are used to investigate their role in salt detection, GCY-22::GFP trafficking towards the cilium and localization to the ciliary tip.
The most prominent candidate interacting protein is GCY-19. GCY-19::GFP is expressed in ASER and colocalized with GCY-22. Loss-of-function of gcy-19 resulted in lower levels of GCY-22::GFP at the ciliary tip. Similarly, gcy-22 loss-of-function animals showed lower levels of GCY-19::GFP at the tip of the ASER cilium. Deletion of gcy-19 did not affect the animals' response to NaCl. We also found GCY-4 and GCY-5 as possible interactors of GCY-22. We detected GCY-4::GFP at the PCMC, but not at the ciliary tip, whereas GCY-5::GFP localised both at the PCMC and the ciliary tip. As rGCs are thought to act as dimers, these findings suggest that GCY-22 might be a common subunit for heterodimeric complexes, possibly to achieve ion-selectivity.
In addition, we identified DAF-25 in our GCY-22::GFP IP-MS experiments. DAF-25 is the ortholog of the mammalian ankyrin repeat and Mynd domain containing protein Ankmy2. DAF-25 has been reported previously to be important for rGC transport. Mutants lacking DAF-25 show no ciliary tip localization of GCY-22::GFP and do not respond to NaCl. Other candidate genes are currently being investigated. This work will allow us to gain insight in the molecular mechanisms that regulate ciliary tip localization of GCY-22.

40. Identifying the GPCRs involved in detecting valproic acid, an anticonvulsant and mood-stabilizing drug, by using C. elegans as a chemosensor

Rogel-Hernandez, Lucero

Valproic acid (VPA) is a short-chain fatty acid derived from the medicinal plant Valeriana officinalis. VPA possesses both anticonvulsant and antimanic properties and has been widely prescribed to treat epilepsy, bipolar disorder, and other neuropsychiatric conditions for decades, but its mechanism of action is not known. Furthermore, prenatal exposure to VPA is associated with birth defects, cognitive deficits, and an increased risk of autism. Thus, a better understanding as to how VPA exerts its therapeutic effects may provide insight needed to develop better therapeutics to promote mental health. To identify the molecular targets of VPA, we are using C. elegans as a chemosensor due to its defined nervous system, well-

characterized chemosensation behaviors, and conserved signaling pathways. In chemotaxis assays, we found that C. elegans are attracted to VPA and this behavioral response is eliminated in animals lacking the AWC chemosensory neurons. Given that chemosensory transduction in these cells commonly depends on G protein-coupled receptors (GPCRs), we determined how VPA attraction is affected in G protein mutants. In this way, we discovered that attraction to VPA is likely to be mediated by a GPCR(s), since egl-30 Gα protein mutants are indifferent to VPA. Indifference towards VPA in this case suggests that in the absence of functional egl-30, upon activation, the GPCR(s) is unable to transduce an intracellular response that eventually leads to attraction. To identify potential GPCR(s) targets for VPA, we looked at the GPCR expression profiles of the AWC neurons in two distinct single-cell RNA seq datasets (CeNGEN, Laurent). From these datasets, we were able to generate a candidate list consisting of 117 GPCRs. We are currently testing the ability of existing candidate GPCR mutant lines to detect valproic acid via chemotaxis assays and are in the process of making additional lines. In the future, once we identified the GPCR(s) involved, we plan to validate whether valproic acid is a direct target for this protein(s) by expressing them in heterologous cells.

41. In situ structure of the SNARE complex in C. elegans

Rosenkranz, Nils; Wieland, Konstantin; Frangakis, Achilleas; Gottschalk, Alexander

Exocytosis is a universal feature of eukaryotic protein trafficking and involves the fusion of vesicles with the plasma membrane. This process is especially important in the nervous system where it regulates neurotransmitter signaling and information transmission. After depolarization of a neuron and Ca2+-influx into the pre-synapse, synaptic vesicles fuse with the plasma membrane and release neurotransmitters into the synaptic cleft. Complexes involving soluble NSF attachment protein receptors (SNAREs) catalyze the formation of fusion pores and drive exocytosis. The aim of the current study is to investigate the in situ structure of these highly conserved SNARE protein complexes in the nematode C. elegans, with the ultimate goal of defining the number of SNARE complexes assembling during vesicle fusion, and their arrangement surrounding the nascent fusion pore. Firstly, we generated transgenic C. elegans strains expressing different fluorescently labelled synaptic markers, which were used to derive embryonal cell cultures. Using the fluorescent signal, we localized the site of neuromuscular synapses and showed healthy growth of neurons and muscle cells in vitro. Moreover, the functionality of neuromuscular synapses under culture conditions was confirmed using an optogenetic approach involving channelrhodopsin expressed in cholinergic neurons. In order to arrest the cells in their physiological state, they were grown on EM-grids and frozen via plunge freezing. Additionally, we also froze L1 stage larvae of the respective C. elegans strains, in which we prepared thin lamellae using cryo-FIB. Using cryo transmission electron microscopy (cTEM) we examined cellular protrusions in culture as well as in L1 staged nematodes and could show a similar cellular behavior, in terms of growth and morphology, under in vitro and in vivo conditions. This highlights the reliability of the cell culture approach. Synaptic terminals and synaptic vesicles can be clearly visualized in cryo-tomograms obtained from cell culture. Currently we aim to quantify tomogram acquisitions of neuromuscular synapses in order to reconstruct a 3D model of the SNARE complex, thus providing in-depth information regarding the formation of fusion pores in C. elegans synapses.

42. Individual behavioral differences in C. elegans

Onoue, Shiori; Kyoda, Koji; Onami, Shuichi

C. elegans can sense many kinds of odorants. The strength of the response varies depending on the odorant and its concentration. However, the response is not 100%, even for a strong odorant. For example, isoamyl alcohol, one of the most robust attractants for C. elegans, attracts more than 90% of the worms but does not about the remaining 10% in the typical experimental settings. Besides, individual worms behave differently even with the same genetic background. It remains unknown in detail how such individual differences in behaviors are created. We try to elucidate the unknown mechanisms that make individual behavioral differences in a “homogeneous” group.
To quantitively analyze individual worm behavior, we developed a new worm tracking system, “Simple Worm Tracker (SWT),” which quantitates worm trajectories automatically from movies. To extract individual behavioral differences from the chemotaxis, we applied SWT to movies of chemotaxis behavior for isoamyl alcohol; a 6 cm plate, every 0.04 s for 50 minutes, was taken using a digital 4K single-lens reflex camera. Then we analyzed these trajectories data how far away from isoamyl alcohol the worms were at each timepoint. Our results suggest they have two phases in chemotaxis behavior; one is approaching the isoamyl alcohol, and the other is moving away or free from it. The timing of switching these phases varied among individuals.
We plan to analyze worm trajectory in more detail, to investigate whether individual differences are a stable and unique property in each worm or a fluctuating property that occurs stochastically.

43. Integrated information assesses the level of consciousness in C. elegans

Ikeda, Muneki; Kato, Saul; Oizumi, Masafumi; Kitazono, Jun

Integrated information is a proposed measure of the level of consciousness exhibited by a complex dynamical system. Estimating integrated information requires fine-grained observation of the complex internal states of the system in question. The advent of single-cell whole-brain recording techniques in model organisms prompts the question of whether integrated information can provide a useful diagnostic of system states that relate to notions of consciousness in different gross organismal states, such as alive, awake, anesthetized, and dead.
Information in this context is defined as Shannon information between the past and present state of a nervous system, and integration is quantified by comparing the information from complete and calculation-procedural partitioned systems. An adequately representative number of patterns of system partitioning must be observed for calculating integrated information, making it currently infeasible to be calculated in higher animals whose brains are composed of over hundreds of millions of neurons.
We calculated integrated information from whole-brain activity in C. elegans, which exhibits transitions between wake and sleep states as well as awake and anesthetized states using our previously published estimation approaches (Hidaka and Oizumi, 2018; Kitazono et al., 2020). In previously published neural activity data of 10 out of 11 worms, we found that integrated information was larger during the wake states and smaller during the sleep states. Also, in previously published neural activity data of 2 out of 2 worms, we found that integrated information was larger during the awake states and smaller during the anesthetized states. We found that the prediction accuracy of consciousness by integrated information was higher

than other information-theoretic measures such as mutual information and transfer entropy. Our results indicate that integrated information is a strong candidate for evaluating the level of consciousness in biological brains.

44. Intergenerational memory of diapause control sensory perception circuitry in a stress dependent manner

Retamales, Esteban; Calixto, Andrea; Serey, Marcela

In nature, environmental conditions shape animal behavior, affect their development, and can alter their evolutionary trajectory. The free-living bacterivore Caenorhabditis elegans can adapt to stressful conditions by exiting reproductive development and entering the stress-resistant dauer larval stage, which can seek improved conditions by stowing onto carrier animals mainly insects. Declining food, high temperatures, overpopulation (pheromones), and pathogens are examples of unfavorable stimuli that promote the worm to diapause. The penetrance in a population of worms that the developmental arrest decision generates varies depending on the stimulus and lacks molecular explanation. It is unknown whether dauers induced by different conditions are the same at a molecular, cellular, or even connectivity level. Moreover, the existence of a memory of diapause in the progeny it has not been described. For example, we show that worms enter diapause at high temperatures (27°C) at ¼ of the population and that this penetrance duplicates ½ in the progeny of diapause parents. In contrast, parents who bypassed the dauer stage (non-dauers) maintain a similar penetrance to their parents. Sensory perception genes (mainly G protein-coupled receptor signaling) are subject to change when comparing adults that experienced diapause (post dauers) vs. non-dauers. We propose to elucidate by RNA-sequencing, dauers P0, dauers F1, and non-dauers F1 induced by temperature (27°C) and overcrowding (pheromone mix) as independent stimuli. Then, explore how the dauer neurons (ASI, ASJ, ASK, ASG and ADF) activity changes between temperature and pheromone dauers and related interneurons (AIA, AIY, and AYZ). Are neurons subject to Chromatin remodeling and regulation of small RNAs?
An enhancement of sensory perception facilitates the finding of more favorable environments to re-enter the reproductive life cycle and could make animals more sensitive to unfavorable conditions making them able to disperse as dauers. Is there a memory of the stimulus that makes their parents hibernate? Does this memory affect their learning outcomes? These questions might explain the critical role of the dauer stage in the evolution of nematodes and how the nervous system participates in generating memory during diapause.

45. Investigating the Diet-Dependent Regulation of Octopamine-Linked Modular Glucosides (MOGLs)

Brown, Tia; O'Donnell, Michael; Dasgupta, Madhumanti

C.elegans likely uses its chemosensory system to locate, discriminate and feed on preferred bacteria. Bacteria produce both attractive and aversive odors that together may signify the identity and value of a particular food source. For example, aversive alcohols such as octanol are produced by certain bacteria and elicit avoidance responses such as reversal behavior. Many bacteria are also capable of colonizing the worm intestine, leading to effects on development, lifespan, fertility and behavior via molecular pathways that are not fully understood. Diet-dependent metabolites such as modular glucosides (MOGLs) - which

comprise a class of N- and O-glycosylated neurotransmitters and neuroactive chemicals - represent promising candidates to regulating the effect of gut microbes on the nervous system. Although these metabolites are diverse, recent work has identified a molecular logic to the assembly of these compounds, enabling mechanistic exploration into their functions. A recently identified, conserved family of carboxylesterase enzymes regulate the specific assembly of acylated MOGLs largely defined by the neuroactive headgroup. One specific family member, the gene encoding CEST-2.1, regulates the assembly of a number of octopamine-linked MOGLs. Octopamine is produced by a single pair of neurons and plays a role in satiety dependent behavioral responses in C. elegans such as octanol avoidance. I have found that upon exposure to 30% octanol cest-2.1 mutant worms exhibit faster reversals than WT, a phenotype similar to mutants unable to produce octopamine. cest-2.1 is expressed in various tissues including the nervous system, indicating that octopamine-MOGLs may play a role in satiety-dependent behaviors. Here I will describe how tissue specific production of octopamine-MOGLs regulates olfactory behavior. These results will inform how a small number of neuromodulator- producing neurons can elicit prolonged changes in behaviors as a function of feeding state. We theorize that modular glycosides may represent a new class of neuromodulators that may be enzymatically controlled to alter nervous system function.

46. Investigating the role of a neural bottleneck in C. elegans

Bonnard, Elsa; Liu, Jun; Scholz, Monika

A neural bottleneck is characterized by the projection of multiple neurons onto a smaller number of neurons. This architecture implies that the network compresses information encoded in the incoming signals. However, it is unclear how biological neuronal networks may perform such compression and how the information content may change through the bottleneck. The RIP neurons in C. elegans represent a simple implementation of a neural bottleneck. They receive converging sensory inputs and provide the only connections to the pharyngeal network controlling pumping, a characteristic muscular motion involved in feeding, through gap junctions with the pharyngeal I1 neurons. To investigate the role of this bottleneck, we supply mechanical stimuli to C. elegans while observing changes in pumping. Estimating information compression requires a large amount of measurements of both the input and output signals. Therefore, we implemented a high-throughput assay to supply substrate vibrations as mechanical stimulus while observing pumping in C. elegans populations. Using our custom image analysis pipeline 'PharaGlow', we can detect automatically pumping events in multiple animals moving on standard cultivation plates (Bonnard and Liu, bioRxiv 2022). Consistent with previous studies, we find that pumping is inhibited by vibrations and this inhibition is abolished in mechanosensory defective mutants. Broadly disrupting the unc-7/unc-9 gap junctions also abolished this inhibition. We will discuss our approach using this experimental paradigm to study sensory information encoding and compression in the RIPs neural bottleneck.

47. Investigating the role of Kinesin-3 motor UNC-104 in regulating the formation and distribution of pre-SVs

Mathew, Amal; Koushika, Sandhya

The precursors of synaptic vesicles (pre-SVs) are formed in the cell body and exit the cell body dependent on the Kinesin-3 motor, UNC-104 in*C. elegans* (1). Synaptic vesicle proteins (SVPs) are thought to be sorted into pre-SVs from an SV-lysosomal intermediate (2). Regulation of size, composition, and recruitment of UNC-104 are key steps in the biogenesis of pre-SVs (3). It is unknown at what step in the biogenesis of pre-SVs, UNC- 104 is recruited and whether it regulates the biogenesis of pre-SVs or merely its transport. To identify compartments where UNC-104 can be recruited, we overexpressed UNC-104 and found that the endolysosomal compartments which were largely restricted to the cell body and proximal neuronal process in wild type were driven into the neuronal process and synapses in *C. elegans* touch receptor neurons. We also observed that the loss of function mutation in the cargo binding domain of UNC-104 restricts the distribution of endolysosomal proteins to the cell body. Together this data is consistent with a model where UNC-104 can be recruited at post-Golgi compartments which are likely intermediate compartments for SVP sorting.
Further, we also observed that UNC-104 overexpression lead to an increase in the size of pre-SVs. This data suggests that UNC-104 might also be involved in regulating the biogenesis of pre-SVs. Together our study identifies novel compartments that are sensitive to the levels of UNC-104 and a novel role of UNC-104 in regulating the size of pre-SVs.
References

1. Hall, D. H., & Hedgecock, E. M. (1991). Kinesin-related gene unc-104 is required for axonal transport of synaptic vesicles in C. elegans. Cell, 65(5), 837-847.
2. Newell-Litwa, K., Salazar, G., Smith, Y., & Faundez, V. (2009). Roles of BLOC-1 and adaptor protein-3 complexes in cargo sorting to synaptic vesicles. Molecular biology of the cell, 20(5), 1441-1453.
3. Choudhary, B., Kamak, M., Ratnakaran, N., Kumar, J., Awasthi, A., Li, C., ... & Koushika, S. P. (2017). UNC-16/JIP3 regulates early events in synaptic vesicle protein trafficking via LRK-1/LRRK2 and AP complexes. PLoS genetics, 13(11), e1007100.

48. Investigation of novel interaction partners to the serotonin-gated ion channel LGC-50

Cesar, Leona Carla; Hardege, Iris; R. Schafer, William; Morud, Julia

Ligand-gated ion channels (LGICs) are present in large numbers in C. elegans. They have been shown to be of high importance for the transduction of sensory information into generating output behavior in animals. One LGIC is the serotonin-gated sodium channel LGC-50, which is expressed in the interneuron RIA, a neuron known to be essential for olfactory aversive learning in C. elegans. LGC-50 has been shown to be recruited to the plasma membrane in a regulated manner after performing aversive olfactory learning, and a 16-amino acid motif in an intracellular region of the protein has been identified to interfere with the regulated membrane trafficking. Similar mechanisms are known from mammalian nervous systems, but in depth understanding is lacking due to the high complexity of these systems and the difficulties in performing genetic perturbations. Using mass spectrometry we have identified the protein NRA-1 as a potential binding partner to LGC-50, this protein appears to bind the previously identified 16-amino acid motif. NRA-1 has previously been shown to be involved in regulation of accurate membrane localization of other ligand-gated ion channels in C. elegans. To validate the interaction between LGC-50 and NRA-1 immunoprecipitation experiments were performed in Xenopus oocytes. The experiments confirmed the binding of NRA-1 to LGC-50 and the interaction was disrupted upon truncation of the suggested binding motif. By understanding the role for NRA-1 in regulated membrane trafficking, we aim to provide a

deeper understanding of receptor plasticity in neural processes and how they are regulated by ligand-gated ion channels.

49. **Kin-recognition mediates social aggregation behaviours in the cannibalistic nematode P. pacificus**

Hiramatsu, Fumie; Lightfoot, James W.

span style="font-family:calibri;">Social behaviours are commonplace across the natural world and range in intricacy from simple aggregation behaviours to more complex interactions. Many of these social interactions depend on the identification of other con-specifics and even kin, nonetheless how this is achieved is often poorly understood. In the predatory nematode Pristionchus pacificus, animals cannibalise other con-specific larvae but their offspring and kin are protected by a kin-recognition system. Furthermore, while most P. pacificus strains are solitary, social behaviours occur in a sub-group of strains. Here, we have explored con-specific interactions between social strains. We have found that social strains of P. pacificus have a strong preference to aggregate with kin, specifically these nematodes group with their own strain and close relatives, and avoid strains that are more distantly related. Moreover, when two distantly-related strains are exposed to each other, one strain frequently dominates and forms aggregates while the other does not. Next, we utilised CRISPR/Cas9 to target a key component in kin-signalling, the hypervariable small peptide SELF-1, to investigate the role of kin-recognition in these social behaviours. Although mutation in the gene self-1 induced a mild self-killing behaviour, this was insufficient to disrupt their aggregation behaviour. Finally, as P. pacificus can be found in the same ecological niche as C. elegans, these two nematodes likely compete with each other for resources. Moreover, wild isolates of C. elegans are also frequently found to be social. Therefore, we investigated how interactions between species affected their sociality and found that the aggregation behaviour of social strains of C. elegans was strongly disrupted by the presence of P. pacificus. Ongoing experiments seek to identify further components that play important roles in kin-recognition as well as to clarify their effects on the aggregation behaviour of these nematodes.

50. **Loss of peptidergic regulation of cholinergic transmission induces postsynaptic homeostatic compensation**

Shao, Jiajie; Liewald, Jana; Gottschalk, Alexander

Neural homeostasis requires proper reciprocal communication between pre- and postsynaptic cells. Different mechanisms are proposed to modulate this bidirectional signaling including transsynaptic adhesion molecules and retrograde signaling. Whether neuropeptidergic signaling regulates this synaptic homeostasis is still largely unknown. In this study, we investigated peptidergic signaling in the context of cholinergic transmission at the neuromuscular junction (NMJ). We have shown previously that neuropeptide signals regulate the cholinergic output at the level of synaptic vesicle (SV) filling. Starting from analyzing the neuropeptide release deficient mutant unc-31, we observed reduced acetylcholine transmission by electrophysiological recording of body muscle EPSCs. However, unexpectedly, we observed enhanced muscle contraction and Ca2+ influx compared to wild-type animals after cholinergic motor neuron (MNs) activation by optogenetics. We propose that in response to reduced cholinergic transmission, postsynaptic homeostasis could compensate

for the reduced input and thus maintain synaptic strength. To test this idea, we directly activated the muscles with channelrhodopsin-2 to in wild-type and unc-31 animals. Muscle activity (evoked muscle contraction) was significantly increased in unc- 31 animals and this phenotype was rescued by restoring UNC-31 cDNA expression in neurons. Several peptide mutants recapitulated the phenotype
of unc-31 and could be rescued by cholinergic MNs' specific expression of respective genomic DNA. Furthermore, we identified a likely modulator mediating the postsynaptic homeostatic response. Future work will focus on understanding the underlying mechanisms of how muscle homeostasis is achieved.

51. **m6A DNA methylation controls forgetting of long-term memories in C. elegans**
Stetak, Attila

Understanding neural circuits and molecular mechanisms underlying changes of synapse strength during learning and memory are the major challenges of neuroscience. While the mechanisms of learning and memory are widely studied, the decay of memories (forgetting), a poorly investigated but apparently highly complex mechanism, is also essential for proper functioning of the brain. Recently, in an attempt to identify genes differentially regulated at the transcriptional level during long-term memory, we identified a core set of genes, out of them nmad-1 represents the C. elegans homolog of DNA m6A demethylase enzyme (Freytag et al., 2017). Using an aversive olfactory associative long-term memory test, we confirmed that nmad-1 gene is indeed transcriptionally upregulated; levels peaking 4h post-training. Furthermore, nmad-1 loss-of-function mutants have surprisingly an increased long-term but not short-term memory performance compared to wild-type worms. Next, we generated a knock-out mutant of the DNA adenosine methyltransferase gene, damt-1, likely acting opposing to the nmad-1 function. As expected, deletion of the damt-1 impaired long- but not short-term memory suggesting that dynamic modification of the DNA m6A modification plays and important role in long-term memory maintenance and elimination. Next, using tissue specific rescue experiments we demonstrated that damt-1 function is required in AVA neuron. Additionally, immunostaining with m6A-specific antibody showed reduced DNA 6-adenosine methylation specifically in AVA neuron upon long-term memory training, while global or AVA-specific RNA methylation levels were not affected. Finally, we compared gene expression differences in wild-type, nmad-1 and damt-1 mutant worms and compared global gene expression profiles and also filtered with the previously defined core memory regulated gene-set in order to identify the methylation regulated memory genes (Freytag et al., 2017). Altogether, our results show that DNA m6-adenosine methylation plays a role in associative long-term memory in worms. Since nmad-1 expression level increases during memory and deletion of the gene results in enhanced memory performance, our findings suggest that this represents a controlled forgetting likely represent an evolutionary conserved forgetting mechanism also present in vertebrates.

52. **Mechanisms by which Innate Immunity Promotes Sleep through Epidermal Antimicrobial Peptides**
Mohsen, Lama; Bringmann, Henrik; Sinner, Marina; Ewbank, Jonathan; PUJOL, NATHALIE; Masurat, Florentin

Wounding and infection trigger a protective innate immune response that includes the production of antimicrobial peptides in the affected tissue as well as increased sleep. Little is known, however, how peripheral wounds or innate immunity signal to the nervous system to increase sleep. We found that, during C. elegans larval molting, an epidermal tolloid/bone morphogenic protein (BMP)-1-like protein called NAS-38 promotes sleep. NAS-38 is negatively regulated by its thrombospondin domain and acts through its astacin protease domain to activate p38 mitogen-activated protein (MAP)/PMK-1 kinase and transforming growth factor β (TGF-β)-SMAD/SMA-3-dependent innate immune pathways in the epidermis that cause STAT/STA-2 and SLC6 (solute carrier)/SNF-12-dependent expression of antimicrobial peptide (AMP) genes. We show that more than a dozen epidermal AMPs act as somnogens, signaling across tissues to promote sleep through the sleep-active RIS neuron. In the adult, epidermal injury activates innate immunity and turns up AMP production to trigger sleep, a process that requires epidermal growth factor receptor (EGFR) signaling that is known to promote sleep following cellular stress. We show for one AMP, neuropeptide-like protein (NLP)-29, that it acts through the neuropeptide receptor NPR- 12 in locomotion-controlling neurons that are presynaptic to RIS and that depolarize this neuron to induce sleep. Sleep in turn increases the chance of surviving injury. Many questions regarding the relationship of sleep and wounding remain unanswered. For example, neuronal EGFR signaling is required for AMP production after wounding in adult worms, but the mechanism of how EGFR controls AMP production is unclear. Also, the molecular mechanisms behind the effects that sleep provides to support survival wounding are unclear. We are hence now working towards a more complete understanding of how sleep and wounding interact at the molecular mechanistic level.

53. Mechanisms of age-related protein aggregation and toxicity

Couzijn, Suzanne; Goya, Eugenia; Janssen, Leen; Seinstra, Renée; Koopman, Mandy; Martineau, Céline; Werkman, Inge; Nollen, Ellen

Protein homeostasis is important to maintain a stable and functional proteome over time. Even though many cellular pathways are involved in maintaining a stable proteome, the capacity of these pathways declines with aging. Proteins harbouring intrinsically disordered regions or low- complexity domains can undergo the phenomenon of liquid-liquid phase separation in which protein droplets are de-mixed from the solution. These droplet inclusions can transition into solid aggregates causing protein toxicity, which is also seen during aging. However, the exact biological mechanisms involved in regulating these phase transitions and driving the associated protein toxicity are still poorly understood. Here we show the potential of a newly developed neuronal Caenorhabditis elegans model that expresses alpha-synuclein in its dopaminergic neurons to unravel the cellular mechanisms of protein phase transition and protein toxicity. Dopaminergic expression of alpha-synuclein seems to result in an age-related increase in inclusions. The biochemical properties of these inclusions can be assessed and monitored during aging using biochemical assays including Fluorescence Recovery After Photobleaching, 1.6-hexanediol dissolvability, and proteinase K digestion. Furthermore, using phenotypic assays we can examine the effect of liquid-like or solid-like inclusions on neuronal function. The characterization of our new alpha-synuclein neuronal C. elegans model will contribute to unraveling the cellular mechanisms underlying protein phase transition and will provide new insights into protein toxicity. We anticipate that our model can greatly benefit both the proteostasis field and the C. elegans field in researching neurodegeneration.

54. MIG-6/papilin and the long-term maintenance of neuronal architecture

Rivollet, Lise; Biard, Marie; Thackeray, Andrea; St-Louis, Philippe; Doitsidou, Maria; BENARD, Claire; Rivollet, Lise; Nadour, Malika

After the initial assembly of the nervous system during embryogenesis, neuronal circuits need to persist lifelong in the face of maturation, growth, body movements, and aging. How neuronal organization is protected throughout life is not well understood. Our research has demonstrated that molecular mechanisms actively maintain the architecture of the nervous system, acting with great cellular specificity (Bénard and Hobert, 2009). sax-7 mutants lack the cell-adhesion molecule SAX-7/L1CAM, and specific neuronal structures that initially develop normally subsequently become disorganized.
Through a genetic screen, we uncovered that loss of mig-6/papilin suppresses neuronal disorganization in sax-7 mutants, suggesting antagonistic roles for these genes: whereas SAX-7 mediates adhesion among neurons, MIG-6 may confer increased flexibility between neurons and their surrounding environment. MIG-6/papilin harbors a papilin cassette, composed of thrombospondin type I and lagrin domains, which is shared with ADAMTS metalloproteinases that remodel the extracellular matrix. In neuronal maintenance, mig-6 functions post-developmentally, and the short isoform of mig-6 is secreted from muscles into the extracellular matrix to non-autonomously impact neuronal maintenance in a mig-17/ADAMTS-dependent manner. Loss of mig-6 leads to the accumulation of extracellular collagen type IV/EMB-9 fibrotic-like structures, which do not occur in the wild type, nor
in sax-7 mutants. Post-developmental depletion of collagen IV reduces these fibrotic-like structures and reinstates neuronal maintenance defects in sax- 7; mig-6 mutants. Moreover, loss of the collagen-IV-crosslinking-extracellular enzyme peroxidasin/PXN-2 also re-establishes sax-7 neuronal maintenance defects in the double mutants sax-7; mig-6, and interestingly, PXN-2 is upregulated in mig-6 mutants. Thus, MIG-6 may ensure a state of flexibility of the extracellular matrix ensheathing neuronal structures that balances neuron-to-neuron adhesion, enabling neuronal architecture to endure lifelong stress. Consistent with this notion, loss of mig-6 bestows enhanced protection of neuronal organization in conditions of increased body movements compared to wild type. Understanding general principles of the maintenance of neuronal architecture and connectivity may help identify key factors influencing the onset and progression of neurodegenerative conditions.

55. Modifying TDP-43 Toxicity

Güngördü, Lale; Koopman, Mandy; Janssen, Leen; Nollen, Ellen; Seinstra, Renée; Hogewerf, Wytse; Wardenaar, Renee; Richmond, Janet; Okerlund, Nathan; Jorgensen, Erik;
Islam, Priota; Brown, Andre

Protein toxicity is thought to underlie several, yet incurable, age-related neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS). TDP- 43 aggregation is the major pathological hallmark of ALS and present in 97% of all cases, suggesting that TDP-43 contributes to the disease mechanism. How protein toxicity triggers cell-and physical dysfunction and leads to degeneration is still not understood. In this project a C.elegans model for TDP-43 induced toxicity is used to study biological mechanisms that lead to disease-related phenotypes. For this aim, we carried out a phenotypic profiling

using the 'Tierpsy 256' dataset and compared TDP-43 worms' behavioral profile to 294 C.elegans mutants, and concluded that cholinergic and GABAergic transmission were the main processes affected. Then we functionally investigated the neuromuscular circuit in TDP-43 worms. Our results showed that we were able to rescue the movement defects in TDP-43 worms with compound treatments and genetic interventions.

56. **Monitoring odor environment to study olfactory learning and navigation in C. elegans**

Chen, Kevin; Gershow, Marc; Wu, Rui; Leifer, Andrew

Animals flexibly adjust behavior in response to environmental contexts and learned experiences. In C. elegans, associative learning with an olfactory cue generates chemotactic behavior towards the cue if it was paired with food [1]. However, it is unknown if olfactory learning modulates sensory-motor processing for a specific navigation strategy, such as the bias in a biased random walk, or alternatively modulates other strategies adaptively during the navigation task [2]. The biophysics with which worms sense airborne cues is also not well understood. Here we investigate butanone-odor associative learning using an odor chamber to precisely measure the odor concentration experienced by worms during odor-guided navigation. We control airborne cues to form a stationary chemical landscape, develop a protocol to calibrate for surface interaction with agar substrate, and measure concentration with an array of digital gas sensors. As worms navigate in the odor environment, we track their trajectories and posture. Preliminary results suggest that navigation strategies can be bidirectionally modulated by olfactory learning. In addition, we investigate behavioral effects of learning using high-throughput assays for optogenetic stimulation. We delivered time varying optogenetic stimulation to populations of freely behaving
C. elegans expressing Channelrhodopsin in the AWC chemosensory neuron. We characterized how temporal properties of signals in the AWC neuron drive the animal to transition into reversal states. We identified temporal integration of these sensory signals over seconds. Consistent with results in the odor environment and previous studies [3], we observe that the animal's reversal behavior becomes more sharply tuned to sensory signals after butanone-odor associative learning. Finally, we propose a statistical model to characterize navigation strategies. This statistical model captures different strategies previously characterized in worms, including biased random walk and gradual change in angle towards higher concentration. Together, we discuss progress towards quantitatively characterizing learned odor-guided navigation with our apparatus, neural perturbation, and a proposed statistical model.

1. Torayama, Takeshi, and Isao. Journal of Neuroscience 27.4 (2007): 741-750.
2. Iino and Yoshida. Journal of Neuroscience 29.17 (2009): 5370-5380.
3. Cho, Christine E., et al. Elife 5 (2016): e14000.

57. **Multigenerational inheritance of beneficial bacterial effects on neuronal integrity and development**

Castillo, Juan Pablo; Caneo, Mauricio; Harcha, Paloma; Zuniga, Rene; Calixto, Andrea

Inherited memory of noxious experiences has been shown to help unexposed progenies avoid similar stressful conditions as well as improve their response efficiency. The nematode C.

elegans is a pioneer model in the study of the underlying principles of such inheritance, observed for bacterial pathogenesis, high temperatures and starvation. However, the inheritance of beneficial life history traits has not been addressed before. We tested whether traits such as neuronal protection and accelerated development offered by GABA and vitamin B12-producing bacteria could be passed through the generations. The inheritance of both traits was studied in animals expressing the MEC-4d degenerin channel in the Touch Receptor Neurons (TRNs) that induces the degeneration of the touch circuit. mec-4d animals were fed with E. coli HT115, P. aeruginosa PAO1 and C. aquatica for 1-3 generations. In each generation, embryos of treated animals were passed to plates with E. coli OP50 and scored for the desired trait. Neuronal protection on animals exposed for two generations to E. coli HT115 and C. aquatica lasted for 2 generations of progeny after the removal of bacteria. However, the exposure of one generation to P. aeruginosa, was sufficient for multigenerational inheritance. Accelerated development followed a similar dynamic to the neuroprotection. Because, both P. aeruginosa and C. aquatica produce vitamin B12 and silence the acdh-1 enzyme, we asked whether the expression of this protein could be affected by multigenerational inheritance. Animals fed with C. aquatica did not express acdh-1::gfp, and E. coli fed animals expressed it maximally in all the intestine. Embryos transferred from C. aquatica to E. coli have a patched pattern of acdh-1::gfp that is restricted to the first two intestinal cells and the posterior part of the intestine, showing that beneficial traits may be associated with metabolic effects that can be inherited.

58. Neurogenetic Mechanisms Underlying Sexually Dimorphic Behavioral States in C. elegans

Reilly, Gregory; Portman, Douglas; Bainbridge, Chance

The ability to flexibly navigate an environment is imperative for an organism's survival. This plasticity in motor behavior requires an animal to integrate environmental stimuli as well as the internal state of the organism itself. In C. elegans, previous work has shown that animals will switch between stereotypical forms of locomotion known as locomotor states depending on both the environment around them and their internal states. Generally, on a patch of bacteria, a hermaphrodite will stochastically switch between two locomotor states: roaming and dwelling. Furthermore, the amount of time spent in each state can be altered by various perturbations to both the environment and internal condition of the organism. However, little is known about how the internal state of genetic sex affects these locomotor states. Studies from our lab have shown that genetic sex influences both body posture and locomotion rate and work from other labs has suggested that male locomotor states may differ from those of their hermaphrodite counterparts. Using a custom Hidden Markov Model analysis, we have found evidence that suggests males, like hermaphrodites, have two locomotor states, but the dynamics of male locomotor state are sex specific; males locomotor states have a unique distribution of linear speed and angular velocity when compared to hermaphrodites. Using tissue specific sex reversal, we aim to determine where genetic sex may be acting in order achieve these sex specific locomotor states. Furthermore, the neurochemical modulators of locomotor states are well defined in hermaphrodites (PDF signaling and serotonin) but poorly defined in males. Regulation of these modulatory pathways could bring about sex differences in locomotor states. Using a combination of mutants and tissue specific knockouts, we aim to determine whether neurochemicals have a sex specific function and, if so, where they might be acting within males. Our results will give further insight into how genetic sex can tune

neural circuitry to achieve sex specific behaviors and more broadly give insight into the complex interplay between genetics, neural circuitry, and behavior.

59. **Neuronal Control of Mitochondrial Stress Responses and their Impact on Metabolism**
Cornell, Rebecca; Handley, Ava; Pocock, Roger

Mitochondrial stress responses are essential molecular mechanisms that maintain homeostasis when an organism is faced with stress. Local stress responses can be communicated to distal tissues to enable global reactions to challenges, thereby increasing the chance of organismal survival. The nervous system is critical for coordinating systemic stress responses, yet the mechanisms by which this is achieved remain largely unknown. In this study, we aim to generate a more holistic understanding of how the nervous system controls distal mitochondrial function and health.
We have previously shown that the ETS-5 transcription factor functions cell non-autonomously through the BAG and ASG sensory neurons to control intestinal fat storage. Additionally, increased intestinal fat storage in the absence of ETS-5 induces a sleep-like state known as quiescence. As sleep and metabolic alterations are a hallmark of systemic stress responses, the combination of increased fat storage and quiescence in anets-5 mutant implicates ETS-5 as a potential stress mediator. Our subsequent analysis revealed that ETS-5 indeed controls a highly specific systemic mitochondrial stress response. Furthermore, we found that the BAG neurons utilise several neuropeptide networks – both dependent and independent of ETS-5 – to illicit unique systemic mitochondrial stress responses. These responses have differing effects on organismal health and mitochondrial function, the full effects of which are being deciphered.

60. **Neuropeptidergic circuitry underlying arousal and sensitization in C. elegans**
Venkatesh, Keertana; Istiban, Majdulin; Chew, Yee Lian; Beets, Isabel; R. Schafer, William

Survival of every organism depends on its ability to modulate behaviour in response to external stimuli. As a consequence, animals can switch between alternative behavioural states, one of which is arousal. A fundamental aspect of arousal is cross-modal sensitization, which primes specific brain circuits for increased vigilance following an arousing, often aversive stimulus. Although observed in most animals, from worms to humans, the molecular factors underlying sensitization and their mode of action in arousal circuits remain elusive.Neuropeptides, which often signal between neurons unconnected by synapses, play key roles in modulating such behaviors across species. The mechanisms by which such wireless neuromodulatory networks control behavior and interact with wired circuitry are less well understood. Using the well-characterized C. elegans nervous system, we
are investigating neuropeptide genes and their roles in arousal circuits. Earlier work has illustrated thatexposure to a prior aversive mechanosensory stimulus (arousing stimulus) results in a heightened response to a subsequent aversive chemosensory stimulus (sensitization). Neuromodulators, in particular neuropeptides, have been seen to play an important role in mediating such a behavioural response. In order to gain insight into the underlying neuropeptidergic circuitry governing this behaviour, we are studying the role of two candidates, FLP-7 and NPR-13, which are counterparts to the human RFamide neuropeptide and prolactin-releasing hormone receptor families, respectively. Behavioral

paradigms have been established to test flp-7 and npr-13 loss-of-function mutants for responses to arousing stimuli. Furthermore, using optogenetic and calcium imaging tools we will dissect the underlying peptidergic circuit and assess neural activity in response to external stimuli in vivo. This will broaden our understanding of the basic principles underlying arousal and sensitization.

61. pOpsicle: An all-optical reporter system for synaptic vesicle recycling using pH-sensitive fluorescent proteins and optogenetic stimulation

Seidenthal, Marius

pH-sensitive fluorescent proteins are widely used in various model organisms to study synaptic vesicle (SV) fusion and recycling. When targeted to the lumen of SVs, these proteins are quenched due to acidification, which is essential for filling SVs with neurotransmitter. Activation of synaptic transmission causes SV fusion with the plasma membrane and exposition of these fluorophores to extracellular neutral pH resulting in an increase in the emitted fluorescence. SV fusion, recycling and acidification can thus be tracked by tagging integral SV proteins with pH-sensitive proteins. Previous work demonstrated that synaptic transmission and SV recycling in sensory neurons could be estimated via the green-fluorescent pHluorin (PMID: 29160768). However, while sensory neurons can be stimulated by application of the respective stimulus, this is not possible in motor neurons. To investigate the refilling of SV pools in this neuron type, we combined pHluorin based probes with the novel red-shifted ChrimsonSA channelrhodopsin. pHluorin was inserted into a luminal loop of the integral SV protein synaptogyrin (SNG-1) and co-expressed in cholinergic neurons with ChrimsonSA. We could show that ChrimsonSA is capable to efficiently depolarize motor neurons in response to red-light (620 nm) while showing no activation at wavelengths needed to excite pHluorin (460 nm). We observed an increase in pHluorin fluorescence during red light illumination, but only when animals were supplemented with the ChrimsonSA chromophore, all-trans retinal. This increase is smaller in snb-1 (synaptobrevin) SV fusion mutants, and in mutants of known SV recycling factors such as UNC-26 (synaptojanin), the decay of pHluorin fluorescence (i.e. re-acidification) after stimulation is delayed. These results suggest that fluorescence rise and decay are indicators for SV fusion and recycling. We will use this all-optical approach 'pOpsicle' (**p**H-sensitive **op**togenetic reporter of synaptic ve**sicle** recycling) to investigate putative SV recycling factors. A similar approach, combining the blue-light sensitive ChR2 and the red-fluorescent pH sensitive protein pHuji will also be described.

62. pprp-1 regulates the state of the synaptic vesicle cluster through phosphorylation of snn-1

Soler García, Miguel

Synapses contain dozens to hundreds of thousands of synaptic vesicles (SV), all grouped in a cluster and maintained by a molecular glue that include Synapsins. Here, we show that pprp-1, an orthologue of the mammalian PHACTR family of PP1 regulatory proteins, regulates Synapsin (SNN-1) phosphorylation. We observe changes in SV cluster size and morphology in pprp-1(lof and gof) correlating with SNN-1 Serine 9 phosphorylation state and to SNN-1-YFP distribution at synapses. Several endocytic proteins are found within the SV cluster, where their function is unclear. Interestingly, we observe mislocalisation of the endocytic protein

ITSN-1-GFP in pprp-1(gof) as well as in snn-1(S9A). Although, the synaptic vesicle (SV) cluster is thought of as a reserve pool participating in neurotransmission, SV cluster may also act as a buffer for the soluble proteins involved in neurotransmission. Our results suggest SNN-1 phosphorylation state might control this buffer.

63. Predicting the regulatory landscape of activity-dependent gene expression in the AFD thermosensory neurons

Bates, Samuel; Harris, Nathan; Bernstein, Matthew; Sengupta, Piali

The AFD thermosensory neuron pair conveys information about environmental temperature stimuli to the organism to inform its behavior. This information relay is accomplished by an increase in intracellular cGMP and calcium levels in the neuron upon exposure to a rising temperature above an experience-dependent sensory response threshold. It has been previously shown that activation of AFD induces changes in its gene expression state, and that these changes are, in part, at the level of transcription. Recently, our lab has described the gene expression changes in AFD in response to temperature using AFD specific TRAP RNA-Seq (see abstract by Nathan Harris). These experiments have identified genes whose expression levels are altered on different timescales following a temperature shift. Moreover, the expression levels of different gene subsets appear to report both absolute temperature and the magnitude of the temperature change, suggesting complex regulatory mechanisms of temperature-regulated gene expression. To begin to identify those transcription factors (TFs) which mediate activity-dependent changes in AFD gene expression, we analyzed the genomic regions upstream of the temperature-regulated genes in AFD identified by TRAP RNA-Seq. We used Simple Enrichment Analysis (SEA) from the MEME suite of DNA sequence analysis tools, together with 2173 experimentally determined TF sequence binding preferences provided by the CIS-BP database, to identify TF-binding motifs enriched in the upstream genomic regions of temperature-regulated genes as compared to AFD-expressed genes whose expression is temperature-independent. This approach generated a list of 57 TF-binding motifs that are over-represented in the upstream regions of AFD-specific activity-regulated genes. Hierarchical clustering of these enriched motifs revealed 27 “families” of related TF motifs. The largest families include motifs representing binding preferences of classical regulators of activity-dependent gene expression such as ATF/CREB family TFs. In initial analyses, we have verified that the expression of a subset of activity-regulated genes is affected in crh-1 CREB mutants. To improve our power to predict and analyze regulators of activity-dependent transcription in AFD, and to describe the entire landscape of temperature-regulated gene expression, we plan to next compare RNA-Seq profiles with activity-dependent changes in chromatin accessibility via ATAC sequencing using affinity purified AFD nuclei.

64. Protein Interactions Underlying Long-Term Memory

Daigle, Kevin; Saifuddin, Mashel Fatema A.; Miller, Julia M.; L'Etoile, Noelle

As the world’s population ages, neurodegenerative diseases pose one of the largest burdens on healthcare and human resources. One predominant and possibly most troubling feature of most neurodegenerative diseases is the loss of memory, yet major molecular processes underlying memory formation are still unknown. By understanding how the key molecular players required for learning and memory function during these processes, we hope to reveal

what molecular oddities might limit the neuroplasticity required for memory formation and consolidation.
OSM-9 is a TRPV channel protein found in roughly 20 sensory neurons within the nematode, Caenorhabditis elegans (Benedetti et al. 2021) and is an ortholog of TRPV5/6 in humans. OSM-9 is important for memory formation and consolidation (Benedetti et al. 2021), but the interactions expected to be part of the memory storage process are currently unknown. OSM-9 has ankyrin repeats that, when disrupted, perturbs the worm's ability to keep memory (Colbert et al. 1997). Worms in our Long Term Memory Assay are able to keep memory after three cycles of being starved in the presence of an attractive odor then fed, however, the ankyrin mutants are unable to form even one cycle memory (Colbert et al. 1997).I hypothesize that OSM-9 promotes long-term memory by interacting with other TRP channel subunits and proteins that bind to the ankyrin repeats. I propose to identify these interacting proteins by immunoprecipitating OSM-9 and using Mass Spec to identify these interactors. I will test which interacting proteins are required to work with OSM-9 in memory formation by knocking each out and asking if that blocks long-term memory. If these proteins have known functions, this may reveal that OSM-9 might perform a similar function to promote long-term memory. Because TRP channels are implicated in human memory and neurodegeneration, these findings may set the stage for therapeutic targets to combat neurodegenerative diseases, such as Alzheimer's disease.

65. **Rapid response to hypoxia in C. elegans is modulated by cGMP signaling**
Zhao, Lina; Fenk, Lorenz; Nilsson, Lars; Amin-Wetzel, Niko; Ramirez, Nelson

The ability to detect and respond to acute oxygen (O2) shortages is indispensable to aerobic life. The molecular mechanisms and circuits underlying this capacity are poorly understood. Here, we characterize the rapid responses of *C. elegans* to the low levels of O2. We found that cGMP signaling plays a modulatory role in acute responses to low O2 by regulating neuroendocrine secretion, and we discovered the guanylate cyclase required for this response. Optogenetic manipulation of cGMP mimics genetic mutants in acute responses to O2 variations. Ca2+ imaging reveals that low O2 stimulus evokes a Ca2+ decrease in several neurons, but we did not find any neurons with increased Ca2+ transient. Our results implicate precise regulation of intracellular cGMP in acute responses to low O2 by *C. elegans*

66. **Rescuing the paralysed phenotype of unc-18 e81 null mutant C. elegans**
Afzal, Khoula; Barclay, Jeff

Mutations in STXBP1/Munc18, an essential protein for synaptic vesicle exocytosis linked to early infantile epileptic encephalopathy, cause protein instability and a reduction in protein expression. Null mutations in STXBP1/Munc18 and its orthologs in flies (rop) and yeast (Sec1) are lethal; however, null mutations in the C. elegans ortholog (unc-18) are viable, but severely paralysed. To identify genes that can compensate for a lack of functional UNC- 18 protein, we performed an unbiased forward genetic suppressor screen on the unc-18 e81 null allele. From this screen a novel strain, unc-18 Rescue, was identified in which the paralysed phenotype of the unc-18 null mutant was reversed to wild-type levels. Whole genome SOLiD sequencing identified two novel mutations in the unc-18 Rescue strain: a nonsense mutation in the kinase domain of a neuronal diacylglycerol kinase (dgk-1) and a missense mutation in the WDR81

orthologue sorf-2. dgk-1 catalyses the phosphorylation of diacylglycerol (DAG) and functions in synaptic transmission by regulating activation of protein kinase C and the synaptic vesicle priming factor Munc13/unc-13. sorf-2 is predicted to function in intracellular trafficking. In this study we confirm that unc-18 Rescue worms are significantly better at locomotion and in electropharyngeal recordings compared
to unc-18 null mutants and are statistically indistinguishable from wild-types. Transgenic expression of dgk-1 in unc-18 Rescue worms reversed the improvement in locomotion to unc-18 null levels suggesting necessity of the dgk-1 mutation for the rescued phenotype. Elevating DAG levels genetically and pharmacologically in unc-18 null mutants failed to improve locomotion, suggesting the additional requirement of the sorf-2 mutation. Heat-shock-induced expression of wild-type sorf-2 in unc-18 Rescue mutants significantly reduced locomotion, confirming the necessity of the sorf-2 mutation.
Finally, recreating the effects of the two novel mutation through sorf-2 RNAi and drug-induced dgk-1 inhibition on unc-18 null mutants resulted in a significant increase in locomotion, confirming the involvement of the two mutations in the rescue phenotype. Further investigation into the sorf-
1 mutation will lead to a better understanding of the mechanisms through which the two mutations bypass the requirement for unc-18 in synaptic exocytosis.

67. **Revisiting the role of the touch receptor neurons in locomotion**
Pidde, Aleksandra; Krieg, Michael

Oscillatory activity underlies the functioning of arguably any living organism. Understanding the principles of cellular oscillators, interactions within the cellular networks and their coupling with sensory feedback in simple organisms, may help advancing the understanding of the pacemakers in more complex vertebrates. Despite several decades of research, it is still unclear where the *C. elegans* traveling-wave sinusoidal locomotion body pattern originates. In particular, the spectrum of models have been debated: on one end the undulations of the head are followed by wave propagation down the body, and on the other, centrally coordinated, multi-cellular oscillations initiate in the units distributed along the body. Our attention was drawn by mechanosensory defective (*mec*) mutations, that apart from being touch insensitive, are also known to be lethargic. We thus hypothesized a role of touch receptor neurons in pacemaker for spontaneous locomotion of *C. elegans* .
In our research, we apply various neuromodulation techniques e.g. tetanus toxin, histamine-gated chloride channels, glutamate mutations, and optogenetics in order to investigate the role of touch receptor neurons in spontaneous locomotion. Our study suggests the involvement of extrasynaptic vesicle-release based mechanism, independent of glutamate communication, downstream from TRNs for neural activity modulation. This evidence may hint towards the involvement of neuropeptides, however disrupting the neuropeptide system, that have been implicated earlier in the sensory and locomotory arousal, was not able to entirely explain the lethargus. Moreover, our preliminary data of spontaneous calcium activity in ventral cord motorneurons of *mec* mutants and wild type nematodes indicated plausible differences in activity between 0.03-0.3 Hz, which may suggest a possible involvement of TRNs in modulation of motorcircuit activity. Our data hints towards a previously unrecognized role of the touch receptor neurons in crawling locomotion similar to plantar mechanoreceptors in bipedal locomotion.

68. Role of GLR-1 In Age Dependent Memory Decline

Gharat, Vaibhav

Normal aging is often accompanied by a deterioration of cognitive functions, including memory decline which can decrease functional independence of individuals and increase the risk of Alzheimer's Disease. In a society with a growing elderly population it is of increased relevance to understand the impact of aging on cognition in order to find strategies that can prevent or limit the loss of cognitive functions in advanced age.

Age-dependent memory decline is associated with structural and functional changes in the brain, including alterations in neuronal structure, loss of synapses and decreased plasticity. Studies in vertebrates have previously highlighted the role of dysregulated glutamate receptor signalling in age- related plasticity decline (Jurado S. 2018). However, the mechanisms regulating the abundance, distribution and properties of AMPA-type glutamate receptors are still largely unknown.

We have previously shown that the AMPA-type glutamate receptor, GLR-1 in C. elegans specifically knocked down in AVA interneurons results in impaired memory (Vukojevic et al. 2012). In the current study, we found that the abundance of total GLR-1 receptors and membrane bound GLR-1 receptors decreased significantly with age in the AVA interneurons of wild type worms which also correlates with age dependent memory decline. On the contrary, mutants with steady memory performance with aging, msi-1(lf) and nhr-66(lf) (Hadziselimovic et al. 2014; Fenyves et al. 2021), did not show age dependent decline in GLR-1 abundance. We also demonstrated that the dynamics of the GLR-1 turnover measured with FRAP reduced significantly with age in wild type animals as compared to mutants with increased memory performance. Finally, to link the decreased GLR-1 abundance with the decreased receptor dynamics and age-dependent memory decline, we are currently investigating these processes in a mutant that expresses a ubiquitination defective variant of GLR-1.

Taken together, in this study we identified changes in abundance and dynamics of the AMPA-type glutamate receptor GLR-1 as potential mediators of decreased plasticity and age-dependent memory decline. With these new insights we hope to contribute to a better understanding of the molecular and structural correlates of age-dependent cognitive decline and, ultimately to unravel novel treatment targets.

69. Role of MADD-4/Punctin processing at the neuromuscular junction

Cizeron, Mélissa; Bessereau, Jean-Louis; Granger, Laure; Romatif, Océane; Vachon, Camille

In C. elegans, body-wall muscle cells receive both excitatory (cholinergic) and inhibitory (GABAergic) inputs. MADD-4/Punctin is an extracellular matrix protein, secreted by motoneurons, which specifies the type of postsynaptic receptors to cluster at each type of neuromuscular junction (NMJ). Two forms of MADD-4 protein are expressed: i) long isoforms (MADD-4L) are expressed by cholinergic motoneurons and are required for the correct localization of cholinergic receptors; ii) the short isoform (MADD-4S) is expressed at both types of synapses and is required at GABA synapses to recruit GABA receptors. It is also secreted at cholinergic synapses where it prevents the inappropriate recruitment of GABA receptors by MADD-4L.

We recently observed that MADD-4 isoforms are cleaved and generate fragments that differentially localize at GABAergic and cholinergic synapses. Our preliminary results suggest that each fragment contributes to the localization of different postsynaptic receptors. Two cleavage sites are detected in MADD-4: one in the linker between the thrombospondin repeat domain (TSR) 4 and TSR5 and one in the linker between the immunoglobulin-like domain and TSR8. Thus, the cleavage of this synaptic organizer increases molecular diversity and may contribute to the fine specification of postsynaptic domain identity. Additionally, we are currently investigating what molecular cues are necessary to stabilize MADD-4 fragments at distinct synapses using genetic screens.

70. Roles of sax-7 in the long-term maintenance of neuronal architecture

Pascal, Marin; Rivollet, Lise; BENARD, Claire

Whereas remarkable advances have uncovered mechanisms that drive nervous system assembly, the processes responsible for the lifelong maintenance of nervous system architecture remain poorly understood. After its initial establishment during embryogenesis, neuronal architecture persists throughout life in the face of the animal's growth, maturation processes, the addition of new neurons, body movements, and aging. The protein SAX-7, homologous to the vertebrate L1 protein family of neural adhesion molecules, is required for maintaining the organization of neuronal ganglia and fascicles after their successful initial embryonic development. Previous work in the lab generated a null allele that completely removes the 20 kb of the *sax-7* locus (by CRISPR-Cas9), as well as *sax-7S* -isoform-specific alleles. This null *sax-7(qv30)* is more severe than previously described mutant alleles at least in some contexts. The loss of *sax-7S* largely phenocopies the *sax-7* null, consistent with previous rescue results demonstrating that the *sax-7S* isoform is key in neuronal maintenance. This isoform maintains neuronal organization by acting post-developmentally, as temporally controlled larval transgenic *sax-7S* expression profoundly rescues the neuronal maintenance defects of *sax-7* null mutants. Interestingly, most of the protein SAX-7 appears to be cleaved on immunoblots, and we show that these cleaved SAX-7S fragments together, but not individually, can fully support neuronal maintenance in vivo. We are further addressing the role of these cleaved fragments of SAX-7S, including by testing their function by single-copy transgenes (miniMos). We are also characterizing the implication of *sax-7S* in cells neighboring neurons. The information harnessed by studying the conserved protein SAX-7/L1CAM in long-term neuronal maintenance in the worm may help decipher processes that go awry in some neurodegenerative conditions.

71. Selective and controlled transgene integration via fluorescent landmark interference

Malaiwong, Nawaphat; Krieg, Michael

Optimal transgene integration requires efficient screening procedures, known and versatile landing sites and low input material. Here, we piggyback on a large transgenic *C. elegans* resource of >289 fluorescent landing sites from the Mos1 insertion (Frøkjær-Jensen, Nature Methods, 2014) that is available to every researcher. We reasoned that these single fluorescent insertion sites of GFP or tdTomato serve as an efficient integration marker to facilitate the genome editing in *C. elegans* : (1) to target the landing site of single or complex transgenes, (2) to apply the CRISPR-mediated color switch as the new gene-editing marker. To

do so, we identified DNA cutting sites in GFP and tdTomato using CRISPR/Cas9 and screened for a loss of nuclear fluorescent in the progeny. In a second step, we identified homozygous integrant, using phenotypic or molecular indicators, either by microscopy or PCR. The large selection of >289 different loci in the *C. elegans* genome facilitates transgenes introduction into different chromosomes using the crRNA(s) against *tdTomato* and *gfp* genes, while simultaneously supplying the injection mix with the plasmid of interest to form an array. We observed the integration of the transgene in non-fluorescent animals via non-homologous end-joining at the locus of the excised markers. This strategy provided the robust advantages for the integration method with the precise integration sites, the clear-cut trait for candidate screening, less time consuming, and less special expertise. Second, we could switch the GFP emission spectrum (509 nm) into blue emission spectrum (448 nm) and vice versa by introducing a point mutation through the homology repair templates. Using the GFP/P4 color switch from different marker strains as a co-CRISPR marker could avoid the gene proximity compared to the conventional marker locus, *dpy-10* (*cn64*) II. Our work pointed out the simplicity and flexibility of the fluorescent landmark interference as the applicable visual indicators, which could potentially be adapted for the new routine methods in the*C. elegans* research community.

72. SEMI-1 is a neuronal selenium-binding protein 1 ortholog, modulating stress resistance, lifespan and thermotaxis in C. elegans

Gong, Weiye; Köhnlein, Karl; Philipp, Thilo Magnus; Priebs, Josephine; Ohse, Verena Alexia; Guerrero-Gómez, David; Kaether, Christoph; Steinbrenner, Holger; Srayko, Martin; Miranda-Vizuete, Antonio; Klotz, Lars-Oliver

The essential trace element Selenium (Se) acts mainly through its incorporation, as selenocysteine, into selenoproteins, many of which are enzymes involved in the maintenance of redox homeostasis. In comparison to humans, with 25 genes encoding selenoproteins, the selenoproteome of Caenorhabditis elegans, consisting of only TRXR-1, is tiny. In addition to selenocysteine, however, Se can also be present in proteins as selenite, binding cysteine residues in selenium-binding proteins. We have recently identified SEMO-1 as a C. elegans ortholog of human selenium-binding protein 1 (SELENBP1). SEMO-1 is not only a pro-aging factor but also a methanethiol oxidase (MTO) that converts methanethiol to H2S, H2O2 and formaldehyde. We here demonstrate that, in addition to SEMO-1, which is expressed in the hypodermis, C. elegans has a neuronal SELENBP1 ortholog, R11G10.2.
Unlike semo-1, R11G10.2 does not encode an MTO, as demonstrated with heterologously expressed and isolated protein, which will henceforth be referred to as SEMI-1 (SELENBP1 ortholog with MTO inactive). Analysis of semi-1 expression patterns was performed using transgenic strains expressing semi-1 transcriptional and translational GFP reporters; resulting GFP signals suggested exclusively neuronal expression of semi-1; GFP- positive neurons were identified as AFD and BAG neurons.
SEMI-1 affects C. elegans lifespan and stress resistance: similar to SEMO-1, depletion of SEMI-1 increased lifespan by approx. 10 %. Moreover, SEMI-1 deficiency increased nematode resistance to the redox-cycler paraquat. As a measure of senescence, autofluorescence was assessed in SEMI-1- deficient strains, which emitted less fluorescence than wild-type worms of the same age. These data suggest that SEMI-1 deficiency improved the health of C. elegans. The rate of pharyngeal pumping was not affected, excluding dietary restriction as responsible for the positive effects of SEMI-1 deficiency.

On the other hand, we identified beneficial effects of the apparent pro-aging factor SEMI-1 for C. elegans. (1) semi-1(+) significantly enhanced the resistance of worms to selenite. (2) semi-1(+) was required for unimpaired positive and negative thermotaxis navigation behavior. In summary, SEMI-1 is a SELENBP1/SEMO-1 homolog apparently confined to AFD and BAG neurons in C. elegans. It is associated with the regulation of stress resistance and lifespan and contributes to C. elegans selenite resistance and thermotaxis.

73. Shotgun phosphoproteomics identifies calcium/calmodulin-dependent protein kinase I (CMK-1) substrates controlling nociceptive plasticity in C. elegans

Rudgalvyte, Martina; Jordan, Aurore; Hu, Zehan; Stumpe, Michael; Kressler, Dieter; Dengjel, Joern; Glauser, Dominique

Nociception is a highly conserved self-protection system alerting animals of potential damage and underlying different forms of pain in human. Some chronic pain conditions may arise from defective modulation in the nociceptive pathway, including within nociceptors, the primary nociceptive sensory neurons. We use Caenorhabditis elegans as a model due to its ability to detect noxious stimuli, perform avoidance behaviors in the form of stimulus- evoked reversals and adapt to repeated stimuli causing a desensitized, analgesia-like state. The worm ortholog of mammalian CaMKI/IV, CMK-1 (calcium/calmodulin-dependent kinase-1) mediates cellular responses to increased calcium levels and is crucial in nociceptors for this avoidance behavior plasticity. However, the downstream elements of the CMK-1 pathway remain unclear.
To identify direct kinase target candidates, we carried out in vitro CMK-1 kinase assays on both peptides and proteins from total worm isolates via shotgun phosphoproteomics. For in vivo direct/indirect target determination, we performed stable isotope labeling by amino acids (SILAC) followed by high-throughput quantitative phosphoproteomics. We compared the phosphorylated proteins in wild type and cmk-1 null animals, which had been grown on ''light'' 12-carbon and ''heavy'' 13-carbon amino acids respectively. By combining results obtained from these in vitro and in vivo studies, we were able to ascertain CMK-1 phosphorylation consensus and delineate a list of potential CMK-1 targets. Mutants for these candidates were then tested for heat avoidance behavior to determine changes in naive sensitivity to noxious heat and/or adaptation. To quantify heat-evoked reversals in naive animals and animals exposed to repeated stimuli, computer-assisted high-throughput analysis pipeline was used. Whereas wild type animal sensitivity decreased following repeated heat stimuli, some mutants failed to adapt. In conclusion, our study reveals several potential CMK-1 targets that may have an important role in behavioral plasticity.

74. Sleep neuron depolarization promotes protective gene expression changes and FOXO activation

Bringmann, Henrik; Koutsoumparis, Anastasios; Welp, Luisa; Wulf, Alexander; Urlaub, Henning; Meierhofer, David; Boerno, Stefan; Timmermann, Bernd; Busack, Inka

Sleep is an essential state that allows for recuperation and survival processes. Disturbing sleep triggers stress responses that promote protective gene expression. Sleep and its deprivation grossly impact gene expression, but little is known about how normal or disturbed sleep control gene expression. Central to the induction of sleep are sleep-active neurons, which inhibit wakefulness and promote survival. Sleep and sleep-active neurons are highly

conserved. In Caenorhabditis elegans, the sleep-active RIS neuron is crucial for sleep and survival. Here, we show that RIS depolarization promotes the protective gene expression response that occurs during developmental arrest. This response includes the activation of FOXO/DAF-16 and expression of DAF-16 target genes such as HSP-12.6, a small heat-shock protein that is required for starvation survival. Disturbing sleep by mechanical stimulation increases RIS depolarization. RIS activation in turn activates DAF-16 and other genes required for survival. Hence, during normal sleep, RIS depolarization promotes protective gene expression. When sleep is disturbed, protective gene expression gets further increased by raised RIS depolarization. We thus link sleep-active neuron depolarization to protective gene expression changes and suggest that the cellular stress response following sleep deprivation could be understood as a safeguarding process that is caused by the overactivation of sleep-active neurons.

75. Tagging ptl-1 in C. elegans with a split fluorophore as proof of principle for "tiny tagging" authenticity

Witten, Gillian; Doonan, Ryan

Fluorescent proteins serve as a method of visualizing gene expression within living organisms. Knocking in these protein tags via CRISPR gene editing is a more authentic way of observing gene expression and protein behavior than using transgenic organisms. However, fluorescent proteins sometimes cause the gene being observed to become dysfunctional because of their large size. For example, the human microtubule-associated protein, tau, misfolds when tagged with GFP. This results in tau neurofibrillary tangles also observed in Alzheimer's disease. To address this problem, split fluorophores may be used, which are fragments of a fluorescent protein that alone are not fluorescent, but have their fluorescence restored once they spontaneously assemble. The smaller fragment serves as a "tiny tag" for the protein of interest, whereas the larger fragment gets expressed
separately. We have used this approach to study the C. elegans ortholog of human tau, known as protein tau-like 1 (PTL-1). The ptl-1 gene encodes 6 isoforms of PTL-1, known as a, b, c, d, e, and f. Based on unique gene structures, we were able to tiny tag PTL-1 in 3 different ways: (1) isoform a only,
(2) the N-terminus of isoforms a, b, and c, and (3) the C-terminus of all isoforms a-f. ptl-1 mutations in C. elegans can result in a mechanosensation defective (Mec) phenotype due to defective microtubules in touch-sensitive neurons. Thus, we did qualitative micro-assays for anterior and posterior gentle touch mechanosensation to assess whether tiny tags affect PTL-1 function. After determining that there was not a statistically significant difference in between the mechanosensation of the wild type and tagged worms, our PTL-1 tags could potentially be used to observe the mechanisms behind tau neurofibrillary tangle formation to better understand diseases such as Alzheimer's at the molecular level.

76. The ACR-16 acetylcholine receptor clusters at specific neuron-to-neuron synapses

Mialon, Morgane; Pinan-Lucarre, Berangere; Bessereau, Patrash, Liubov; Jean-Louis

C. elegans has proven to be an extremely powerful model to decipher the cell biology of synapses. Despite thousands of synaptic connections described in the whole animal, very few chemical synapses have been investigated at the molecular level. We recently identified

neuron-to-neuron synapses along the ventral cord that can be genetically tracked using a fluorescent reporter of the ACR-16 acetylcholine receptor, ortholog of the human alpha7 acetylcholine receptor. At these synapses, the ACR-16 acetylcholine receptor likely concentrates at postsynaptic sites. We built a transcriptional reporter for acr-16 (Pacr-16::AID-mNeonGreen) that we imaged in the NeuroPAL strain. By combining NeuroPAL imaging and single-cell expression data from the CenGEN consortium, we identified key neurons that express ACR-16 and receive cholinergic inputs in the ventral cord. Consistently, auxin-mediated degradation of an ACR-16-AID-scarlet knock-in reporter specifically in these key neurons robustly decreased ACR-16 synaptic levels. To identify neurons that are presynaptic to ACR-16 clusters, we seek to construct a set of cell specific presynaptic reporters. Furthermore, connectivity data indicates more cholinergic inputs at ACR-16 expressing neurons than ACR-16-AID-scarlet clusters. We speculate that ACR-16 might cluster at a specific subset of cholinergic connections, and that other cholinergic receptors might cluster within the same neuron opposite other presynaptic neurons.

77. **The development of histamine-activated GPCRs as chemical-genetic tools for C elegans**
Pokala, Navin; Patel, Gauri

Neuromodulators such as biogenic amines and neuropeptides regulate every aspect of behavior. Most neuromodulators use G-protein coupled receptors (GPCRs). Being able to activate specific G-protein signaling pathways in neurons of interest would be a powerful tool for studies that aim to understand the architecture of neuromodulation. For this reason, we are developing histamine-activated GPCRs for use in C elegans neurons. HRH1 primarily acts via Gaq, HRH2 via Gas, and HRH4 via Gai. By expressing a particular receptor in cells of interest, we can use histamine to activate specific G-protein pathways in those cells. Histamine is an especially attractive molecule for chemical genetics in C elegans because it is inexpensive, water- soluble, enters worms rapidly, and has no effect on behavior. We previously demonstrated the use of the Drosophila histamine-gated chloride channel HisCl1 for reversibly silencing specific C. elegans neurons. While wild-type HisCl1 has no activity without histamine, the wild-type histamine-activated GPCRs have significant basal activity in the absence of histamine, both in their native organism and in C elegans. To identify receptor variants with lower background activities, we have screened receptor variant libraries in yeast strains in which animal Gα subunits are coupled to the endogenous mating pheromone signaling pathway. We are characterizing improved receptor candidates in C elegans neurons.

78. **The fear of hunger: starvation-induced aversive associative memory in C. elegans**
Blénesi, Szilvia; Gábor, Hajdú; Sőti, Csaba

Maladaptive behaviors are linked to eating and metabolic disorders, but how memories of past starvations shape feeding behaviors is unclear. The roundworm Caenorhabditis elegans with an entirely mapped connectome, learning and memory capacities is a versatile model of neuroscience. It is well-known that nematodes reduce their attraction towards olfactory cues previously paired with starvation. Here, we investigated the mechanism of the starvation-induced behavioral change.
We employed benzaldehyde (BA) and diacetyl (DA), naturally attractive food-derived odorants sensed by different chemosensory neurons. We confirmed that after a 4-hour starvation in

the presence of BA or DA, worms significantly reduced their chemotaxis to both odorants. Starvation alone or the odorant paired with food did not affect naive behavior, excluding olfactory adaptation as a mediator. Further, the persistent change after 24- hour starvation and 4-hour recovery suggested learned alteration and long term memory formation in food searching behavior. Worms also reduce feeding behavior in the lasting absence of food. Strikingly, reduced rate of pharyngeal pumping in the presence of starvation-associated odorants demonstrated a similar learned decrease of feeding behavior when food was available. Conditional inhibition of a single chemosensory neuron with avoidance stripe assays ruled out the possibility of habituation and provided evidence for aversive associative learning.
Our findings reveal a long-term aversive memory in response to starvation which might help avoid impending adversity. We propose that re- encountering olfactory cues associated with previous episodes of starvation might paradoxically elicit avoidant feeding behavior by retrieving the stressful memory.

79. The Hox gene mab-5 generates motor neuron diversity in posterior ventral nerve cord

Prahlad, Manasa

All nervous systems utilize a great diversity of cell types. Classification of neuron types and subtypes generally depend on functional, morphological, and molecular criteria. However, neuronal subtype diversity along the antero-posterior (A-P) axis remains an understudied facet of nervous system development.
C. elegans is a prime model to study neuronal diversity. C. elegans possesses 53 cholinergic and 19 GABAergic motor neurons (MNs), grouped into eight anatomically distinct subtypes. MNs of each subtype are intermingled along the A-P axis of the ventral nerve cord (VNC, analogous to the vertebrate spinal cord). UNC-3 (Collier/Olf/Ebf) and UNC-30 (Pitx) are terminal selectors of cholinergic and GABAergic MN identity, respectively. Both promote the expression of genes critical to neuron function (e.g., neurotransmitter (NT) synthesis, NT transport) in all MN subtypes, irrespective of cell body position in the VNC.
However, unpublished single-cell RNA-seq data generated in collaboration with the Miller lab at Vanderbilt University shows that there is molecular diversity among MNs based on their cell body position; i.e., MNs in the anterior and posterior regions of the VNC form distinct molecular clusters. The gene mig-13, which encodes a protein crucial for the migration of some neuroblasts, provides a starting point to study this diversity: mig-13 is expressed in most MNs anterior to the vulva, but in almost none posterior. Towards elucidating mechanisms of neuronal diversification along the A-P axis, we investigated the regulation of mig-13. We hypothesized that its region-specific expression pattern was due to Hox factor activity. Indeed, in the absence of the posterior VNC Hox factor mab-5 (Antp/Hox6-Hox8), mig-13 was de-repressed in many MNs in that region. We also found that the terminal selectors UNC-3 and UNC-30 activate mig-13 expression in anterior cholinergic and GABAergic MNs. Finally, we hypothesized that mab-5 antagonizes the activator function of terminal selectors in posterior MNs. To test this, we built double mutant strains lacking either terminal selector and mab-5 gene activity. The resulting loss of mig-13 expression in posterior MNs confirmed our hypothesis. Altogether, our findings illuminate how Hox factors intersect with terminal selectors to generate neuronal diversity along the A-P axis of the nervous system.

80. **The mind of a dauer: topological features of the nervous system**

Choe, Daniel T.; Yim, Hyunsoo; Bae, J. Alexander; Nguyen, Ken C.; Bahn, Sang-kyu; Kang, Hae Mook; Hall, David H.; Kim, Jinseop S.; Lee, Junho

The connectome of the C. elegans dauer is hypothesized to have specific topological features due to its developmental and behavioral divergence. To test the hypothesis, we have reconstructed the neural network of the dauer nerve ring from serial section electron microscope images. Using the resources, in this study we compare basic topological properties and motifs of dauer connectome with normal developmental stages, particularly L3 and adult. Overall, the connectivity of dauer increased significantly compared to the case of L3 to the level of adult stage, for both in-degree and out- degree. Further inspections by neuronal classes show prominent connectivity increases for sensory and motor neurons in dauer. Various centrality measures also indicate that connections are denser in sensory and motor neurons. Specifically, betweenness centrality of interneurons show a significant decrease compared to L3. This implies that the interneurons contribute less in neural communication in dauer. Such increase of class-wise connectivity and centrality can be associated with the modularity of sensory and motor neurons in dauer. Indeed, the clustering coefficient of sensory and motor neurons is greater in dauer than in L3. This is supported by two-cell and three-cell motif analyses. Bidirectional connections are significantly overrepresented in dauer, which is consistent with previous studies. Similarly, triangular patterns are more prevalent in three-cell motifs of intrasensory and intramotor connections. In summary, the analyses on topological properties and motifs reveal that the dauer connectome is more modular especially within sensory and motor neurons, which resembles that of adult stage. The altered network connectivity may help dauer to amplify signals within sensory and motor neurons and to directly communicate between sensory and motor neurons. These connectivity changes may enhance prompt motor response to subtle stimuli.

Poster session II

81. The modulation of acid-sensing ion channels (ASICs) during learning

Ferguson, Larissa

During neurotransmission, the release of protons alongside neurotransmitters from acidic vesicles results in an acute local acidification of the synaptic cleft. However, the role of synaptic pH changes in unknown. Acid-sensing ion channels (ASICs) are activated by low pH and are concentrated in synaptically dense regions of the brain (Wemmie et al., 2003) and are required for longterm potentiation in the hippocampus (Wemmie et al., 2002) and learned fear behaviour in rodents (Wemmie et al., 2004), suggesting a role in neurotransmission. In the nematode, Caenorhabditis elegans, ASIC-1 mediates associative learning by modulating neurotransmitter signalling (Voglis & Tavernarakis, 2008). However, the mechanisms underlying ASIC activation and function at synapses to facilitate plasticity during learning are not fully understood. Here, we explore how the pH sensitivity of ASICs is modulated in vivo during learning. Using two-electrode voltage clamp (TEVC), we explore how subunit composition affects gating properties of the channel in a Xenopus oocyte expression model, as well as exploring endogenous neuromodulators that bind to ASICs to affect function. Using TIRF microscopy, we examine subunit composition of membrane-bound fluorescently tagged ASICs in Xenopus oocytes. Future work will explore subunit dynamics in C. elegans in vivo during learning using chemosensory conditioning and food-dependent thermotaxic associative learning tasks. This work will further our understanding of how pH changes affect neurotransmission and elucidate a role for the modulation of acid-sensing channels in learning.

82. The PBAF chromatin remodeling complex is required for cholinergic motor neuron subtype identity

Osuma, Anthony

We have previously shown that the evolutionarily conserved COE (Collier, Olf, Ebf)-type transcription factor UNC-3 acts as a terminal selector and determines cholinergic motor neuron (MN) identity in multiple MN classes (SAB, DA, DB, VA, VB, AS). UNC-3 directly controls the expression of both shared (e.g., acetylcholine pathway genes) and class-specific terminal identity genes (e.g., ion channels, neurotransmitter receptors). However,*unc-3* is expressed in all these MN classes, leading us to hypothesize the existence of repressor proteins that restrict the ability of UNC-3 to activate class- specific genes more broadly. To test this hypothesis, we performed a forward genetic screen using the UNC-3 target gene *glr-4* , which encodes a glutamate receptor subunit selectively expressed in SAB MNs. We found that *pbrm-1* , the sole *C. elegans* ortholog of the evolutionarily conserved chromatin regulator BAF180, selectively prevents expression of a transgenic *glr-4* reporter in DA, VA, and AS classes, resulting in mixed MN identity. Similar results were obtained when we monitored endogenous *glr-4* expression via RNA fluorescent in situ hybridization and a reporter allele. Since PBRM-1/BAF180 is a subunit of PBAF, a chromatin remodeling complex of the SWI/SNF family, we reasoned that animals lacking gene activity for other PBAF subunits might display similar MN phenotypes. We indeed found that loss of *swsn-9* (*C. elegans* ortholog of human BRD7 and BRD9), *swsn-7* (ortholog of human ARID2), and *phf-10* (ortholog of human PHF10) results in gain of *glr-4*

expression in these three MN subtypes. Rescue and RNAi experiments using cholinergic MN-specific promoters (*cho-1* and *lin-39*) further demonstrated that these PBAF components, despite their ubiquitous expression, act cell-autonomously in post-mitotic MNs. Finally, we found that the transcription factors MAB-9/Tbx20 and UNC-4/UNCX repress *glr-4* expression in AS and DA/VA neurons, respectively. To account for the observed specificity of PBAF-mediated*glr-4* repression in select MN classes, we hypothesize that PBAF is recruited by MN class-specific transcription factors (e.g., MAB-9, UNC-4) to repress UNC-3 target genes. Altogether, we
provide novel insights on the epigenetic mechanisms that generate neuronal diversity by uncovering a previously unrecognized, neuron-specific role for the PBAF chromatin-remodeling complex in selective repression of terminal selector target genes.

83. **The regulation of brain-body communication in zebrafish and C. elegans**
Bayer, Emily; Schier, Alex

The regulation of internal organs has been historically considered to be 'self-contained' and autonomous. However, it is increasingly apparent that brain-body communication modulates not just the internal organs, but also the brain itself. To dissect the regulation of brain-body communication, I am combining the specificity of the C. elegans pharyngeal nervous system with study of less-characterized visceral innervation in zebrafish.
In C. elegans, communication between the 20 neurons of the pharyngeal nervous system and the 280 neurons of the somatic nervous system is mediated almost entirely via neuropeptide signaling. I have generated transgenic lines to induce neuronal activation and silencing of pharyngeal neurons in combination with a behavioral screen to identify novel behavioral outputs modulated by pharyngeal sensation. In a more general approach, I am also using an intersectional degradation system to eliminate neuropeptide processing specifically from the pharynx to evaluate whether there are consequences on somatically-generated behaviors.
In zebrafish, visceral innervation is compact enough that it can be detailed comprehensively, but in contrast to the well-characterized pharyngeal nervous system of C. elegans, the genetic identities, modalities, and functions of visceral neurons (from both the vagus nerve and cranial sensory ganglia) are still largely undescribed. I have used scRNAseq approaches to collect transcriptomic data on both of these neuron populations, and will complement this approach with spatial transcriptomics to resolve the anatomical organization of molecular cell types. The combined study of C. elegans and zebrafish will help uncover both general principles and unique aspects of brain-body communication.

84. **The role of OSM-9 and Whole Brain Calcium Dynamics During Aversive Memory Recall**
Saifuddin, Mashel Fatema A.; Dunn, Raymond; Miller, Julia; Chandra, Rashmi; L'Etoile, Noelle; Lin, Christine; Daigle, Kevin

Memory provides an important survival benefit across many species, as failure to recognize signals associated with harmful agents can be deadly. We use a spaced cycle odor-training paradigm to induce learning in C. elegans by pairing butanone with starvation, and this olfactory memory can last over 24 hours. However, the changes in neural dynamics and molecular processes that allow an animal to make the choice to avoid an odor are still unknown.

Mutations that interfere with memory can reveal the cells and processes required for learning and memory consolidation. Prior studies showed that loss of the transient receptor potential (TRP) OSM-9/TRPV5/TRPV6 channel impaired single exposure-induced memory. Here, we report that this TRPV channel is also required for acquisition and possibly consolidation of sleep-dependent, long-term memory of butanone. We found that OSM-9 is expressed in over 20 sensory neurons however, none of these are required for butanone chemotaxis. This presents a quandary: what are the OSM-9 expressing neurons doing during memory consolidation?
First, we asked what calcium activity patterns butanone presentation evokes in the entire anterior ganglion of an animal that is attracted to butanone (buffer-trained control). We then compared that with worms that are indifferent to that odor (butanone trained). We expected that the animals that sought out butanone would show the neural correlates of reduced turns and backing during butanone exposure. By contrast, if the animals have learned to avoid butanone, those animals will increase turns and backing and their neural correlates would reflect that. By asking what neurons are differentially active, this provide us an unbiased approach to identify neurons important to the butanone-indifferent response of an animal that has consolidated memory.
Finally, we will image the entire neuronal ensemble in animals that lack OSM-9. This may reveal new patterns of activity that could explain how loss of OSM-9 can block consolidation. Examining the patterns of activity within the butanone-sensing circuit as well as the interactions with other neuronal ensembles will provide us with important insight into the decision-making process and how it changes after learning.

85. **The terminal selector UNC-3 negatively autoregulates in cholinergic motor neurons**
Destain, Honorine; Kratsios, Paschalis

Terminal selectors are transcription factors that are continuously required throughout life to ensure expression of effector genes that convey appropriate cellular identity and function, termed terminal identity genes. As terminal selectors are powerful inducers of particular cell fates, their expression levels require precise regulation. In many cases, terminal selectors have been demonstrated to positively autoregulate; however, involvement of additional regulatory mechanisms remains unclear. Here, we describe a molecular mechanism for the precise regulation of terminal selectors using the COE (Collier/Olf/EBF) family member UNC-3, the terminal selector of cholinergic motor neurons (MNs) in C. elegans. We have previously shown that the Hox proteins LIN-39 and MAB-5 positively regulate unc-3. Here, we demonstrate that, contrary to most terminal selectors, UNC-3 negatively autoregulates. To probe the mechanism of negative autoregulation (NAR), we analyzed expression of transcriptional reporters in a variety of unc-3 mutants with missense mutations within the DNA binding, Zinc finger, and Immunoglobulin-like/Plexin/Transcription Factor (IPT) domains. We found that all these mutants caused increased expression of unc-3 transcriptional reporters. Our ChIP-seq data reveal UNC-3 binding at its own locus, suggesting NAR occurs via a direct mechanism. We identified multiple UNC-3 binding sites (termed COE motifs) that overlap with UNC-3 ChIP binding peaks upstream of exon 1 and within intron 1, and will mutate them individually in the context of an unc-3::GFP endogenous reporter. We expect that such manipulations will specifically disrupt NAR, and predict according to systems biology literature that unc-3 expression levels will become more variable. To test whether precise levels of UNC-3 are functionally important, we manipulated UNC-3 expression through auxin-induced

degradation and over-expression. Upon UNC-3 depletion, cholinergic MNs lose expression of terminal identity genes. Upon UNC-3 overexpression, cholinergic MNs inappropriately express terminal identity genes normally restricted to a subset of MN classes (ex. DA/DB). Altogether, we find that critical levels of UNC-3 are important to ensure appropriate cholinergic MN fate, and propose NAR as mechanism to ensure robustness of terminal selector gene expression levels.

86. **Toxic stress-specific cytoprotective responses regulate learned behavioral decisions in C. elegans**

Gábor, Hajdú; Blénesi, Szilvia; Sőti, Csaba

Recognition of stress and mobilization of adequate “fight-or-flight” responses is key for survival and health. Previous studies have shown that exposure of *Caenorhabditis elegans* to pathogens or toxins simultaneously stimulates cellular stress and detoxification responses and aversive behavior.
However, whether a coordinated regulation exists between cytoprotective stress responses and behavioral defenses remains unclear.
Here, we show that exposure of *C. elegans* to high concentrations of naturally attractive food-derived odors, benzaldehyde and diacetyl, induces toxicity and food avoidance behavior. Benzaldehyde preconditioning activates systemic cytoprotective stress responses involving DAF-16/FOXO and SKN-1/Nrf2 in non-neuronal cells, which confer both physiological (increased survival) and behavioral tolerance (reduced food avoidance) to benzaldehyde exposure. In contrast, diacetyl preconditioning augments diacetyl avoidance, weakens physiological diacetyl tolerance, and does not induce apparent molecular defenses. The inter-tissue connection between cellular and behavioral defenses is mediated by JNK-like stress-activated
protein kinases and the neuropeptide Y receptor NPR-1. Reinforcement of the stressful experiences using spaced training forms stable stress-specific memories. Memory retrieval by the olfactory cues leads to avoidance of food contaminated by diacetyl and context-dependent behavioral decision to avoid benzaldehyde only if there is an alternative, food-indicative odor.
Our study reveals a regulatory link between conserved cytoprotective stress responses and behavioral avoidance, which underlies “fight-or-flight” responses and facilitates self-protection in real and anticipated stresses. These findings imply that variations in the efficiency of physiological protection during past episodes of stress might shape current behavioral decisions.

87. **TYRAMINERGIC COROLLARY DISCHARGE FILTERS REAFFERENT PERCEPTION IN A CHEMOSENSORY NEURON**

Zimmer, Manuel; Riedl, Julia; Fieseler, Charles

Interpreting sensory information requires its integration with the current behavior of the animal. Yet how motor-related circuits influence sensory information processing is incompletely understood. Here we report that the reversal locomotor state instantaneously modulates the activity of BAG O2 / CO2 sensory neurons. Recording neuronal activity in animals freely navigating CO2 landscapes, we found that during reverse crawling states BAG

activity is suppressed by tyraminergic corollary discharge signaling. We provide genetic evidence that tyramine released from the RIM reversal interneurons extrasynaptically activates the inhibitory chloride channel LGC-55 in BAG. Moreover, we find that LGC-55 signaling cancels out perception of self-produced CO2 and O2 stimuli when animals reverse into their own gas plume in ethologically relevant aqueous environments. Disrupting this pathway genetically leads to excessive behavioral responses to acute CO2stimuli and affects normal CO2 perception upon initiation of forward crawling. Our results show that sensorimotor integration involves corollary discharge signals directly modulating chemosensory neurons.

88. **Uncovering new GABA transporters in C. elegans thanks to an atlas of amino acid transporter expression**

Samba, Nalia; Tissot, Charlotte; Cheynet, Elise; Gendrel, Marie

Functional neuronal circuits rely on a combination of both excitation and inhibition. In mature neurons, the main inhibitory neurotransmitter is GABA. Traditionally, neurons have been classified as GABAergic if they co-expressed three protein determinants: 1) GAD/UNC-25, which synthesizes GABA from glutamate, 2) VGAT/UNC-47, a vesicular transporter that packages GABA into synaptic vesicles, and 3) GAT/SNF-11, a plasma membrane transporter that recaptures extracellular GABA. In C. elegans, only 26 out of 302 neurons were considered GABAergic, until an improved immunostaining enabled the identification of 15 additional GABA-positive neurons. Although they stain for GABA and contact neurons expressing post synaptic GABAA receptors, they do not all coexpress GAD/UNC-25, VGAT/UNC-47 and GAT/SFN-11. In particular, three pairs of neurons express none of those. They are thus unable to synthesize, uptake or package GABA the way we know it.
We hypothesize that those neurons use unidentified transporters for GABA uptake and vesicular packaging. The present work aims to identify them, and in particular to probe 51 putative amino acid transporters belonging to the SLC6 (GAT/SNF-11's family), SLC7/3, SLC16 and SLC36/38 families. In
C. elegans , most of these transporters are not functionally characterized yet. Thus, as a first step, we undertook to determine the expression pattern of the candidates. So far, using a fosmid-based reporter strategy (Tursun *et al* , 2009), we generated transgenic lines for 13 genes out of the 51 putative amino acid transporters. Neuronal expression could be detected in 8 out of the 13 genes.
This reporter strategy combined with anti-GABA immunostaining will enable us to assess if the transporters are expressed in our three pairs of neurons of interest. If so, we will then further characterize them in order to determine if they are indeed transporting GABA.
Additionally, thanks to the NeuroPAL strain (Yemini et al. 2021) and other cell specific markers, we will establish a map of the potential expression sites of the 51 putative amino acid transporters.

89. **Uncovering new GABA transporters in C. elegans using classical immunostaining and a GABA sensor strain**

Cheynet, Elise; Samba, Nalia; Gendrel, Marie

Functional neuronal circuits rely on a combination of both excitation and inhibition. In mature neurons, the main inhibitory neurotransmitter is GABA. Traditionally, neurons have been classified as GABAergic if they co-expressed three protein determinants: 1) GAD/UNC-25, which synthesizes GABA from glutamate, 2) VGAT/UNC-47, a vesicular transporter that packages GABA into synaptic vesicles, and 3) GAT/SNF-11, a plasma membrane transporter that recaptures extracellular GABA. In C. elegans, only 26 out of 302 neurons were considered GABAergic, until an improved immunostaining enabled the identification of 15 additional GABA-positive neurons. Although they stain for GABA and contact neurons expressing post synaptic GABAA receptors, they do not all coexpress GAD/UNC-25, VGAT/UNC-47 and GAT/SFN-11. In particular, three pairs of neurons express none of those. They are thus unable to synthesize, uptake or package GABA the way we know it.

We hypothesize that those neurons use unidentified transporters for GABA uptake and vesicular packaging. The present work aims to identify them, and in particular to probe 51 putative amino acid transporters.

Here, we present a part of this project, where we analyze how null alleles of these genes affect GABA localisation. Indeed, if the neurons of interest rely on a transporter to uptake GABA or to load it into vesicles, removing this protein should suppress staining of these neurons or increase it, respectively. We have a collection of 26 mutant strains. These strains are currently being characterized using anti-GABA immunostaining. For those with no null allele available, generation of some of them using Crispr/Cas9 technology is under progress.

Additionally, we are developing a strain with expression of a GABA sensor in neurons. This should allow to detect GABA more easily and more reliably, as well as to study how its localisation is affected by several conditions in living nematodes. Transgenic lines were obtained, and neuronal fluorescence can be seen. Undergoing integration will allow to further characterize it.

90. Using Caenorhabditis elegans to explore the mechanisms of 3D collective animal behaviour

M. Perez, Daniela; Ding, Serena

The versatile model organism, Caenorhabditis elegans, exhibits various collective behaviours such aggregation, swarming, dynamical network formation, and towering. Although some of these behaviours have been known for decades, comprehensive quantitative studies have only begun more recently and have thus far been restricted to 2D behaviours. However, 3D behaviours such as towering (collective nictation) are not only fascinating to observe but also critical for the ecology in a wide range of nematode species, as it is understood to be a dispersal mechanism integral to their boom- and-bust lifestyle. We proposed to characterise towering behaviour in C. elegans under controlled laboratory conditions, using a combination of classical behavioural ecology methods and novel imaging and tracking tools. We first identify conditions that robustly promote towering in a Petri dish, and then quantitatively assess worm locomotion and interactions in the 2D plane as individuals enter and exit the tower in the third dimension. We also plan to manipulate sociality using social and asocial strains, to unveil if the propensity for social interactions previously found in 2D is generalisable to the 3D perspective. By seeking a detailed characterisation of the behaviour and identifying cues for coordinated group decision-making, this study opens doors for future assessments of the mechanism and the function of this important collective behaviour.

91. **Video processing of whole brain activity recordings in freely moving in C. elegans**
Fieseler, Charles; Hille, Lukas; Rey, Ulises

Recent neuroscience has seen various paradigm shifts due to the availability of large-scale neuronal activity datasets obtained by multi-electrode arrays or Ca2+-imaging techniques. In the nematode *C. elegans* it became possible to image the head ganglia at single-cell resolution in freely moving animals. We have established a microscopy pipeline based on a speed-optimized spinning disk microscope that enables worms to crawl normally and to engage in goal-directed behaviors such as food-chemotaxis during imaging sessions. However, these image datasets represent a challenge for generating artifact-free time series data. This is due to low signal-noise ratios when imaging with tolerable laser excitation-intensities, short exposure times to avoid motion blur and non-rigid tissue movement while worms engage in their undulatory gait. To overcome these challenges and to process these videos, we focused on the two critical steps: segmentation and tracking. Recent progress in image processing has been dominated by artificial neural networks, which, in the right circumstances, dramatically outperform all classical methods. Collecting a large amount of training data is the most costly ingredient in these methods, and to do this a custom annotation and visualization GUI was built. For both technical challenges we modify previously published network architectures. For segmentation, we train a StarDist neural network that uses a star-convex shape prior to segment densely packed neurons. For tracking, we use a SuperGlue network, which uses attention-based graph neural networks to leverage global-image information to match objects across separate images. Segmentation performance was compared ground truth annotations by multiple human annotators, and achieves close to human accuracy, and approximately 80% of neurons are reliably segmented across time. Tracking performance of these reliably segmented neurons was compared to published work, and achieves 99% accuracy when compared to ground truth manual annotation, outperforming recently published tracking methods in *C. elegans* by 10-20 percent. Finally, this work would not be possible without a cohort of student workers doing manual annotation

92. **Visualising neuropeptide spatial range of action within the nervous system**
Goss-Sampson, Eve; Barrios, Arantza; Jimeno-Martín, Ángela; Beets, Isabel; Watteyne, Jan

Past experiences, moods and emotions change our behaviours, partly due to neuromodulation of underlying circuits by neuropeptides. Neuropeptides are secreted, diffusible proteins that act on G-protein coupled receptors (GPCRs), providing long-lasting modulation to hard-wired neuronal circuits. Many neuropeptide sources are not synaptically connected to their targets, thus for a neuropeptide to elicit a given function, the signal may rely on extrasynaptic diffusion towards the specific target(s). However, the distance neuropeptides can diffuse and the regulatory mechanisms underlying target specificity are unknown.
We are employing C. elegans to establish an in vivo system to visualise neuropeptide based neuronal communication to use as a tool to identify molecular and cellular mechanistic regulators of neuropeptide diffusion. We are focusing on Pigment Dispersing Factor-1 (PDF-1) and its receptor PDFR- 1, a highly conserved neuropeptide that in worms is involved several behaviours, including exploration and associative olfactory learning. By harnessing GPCR signalling cascades, we have introduced a synthetic pathway that converts the interaction

between PDF-1 and PDFR-1 into a fluorescent signal, resulting in a basal activation pattern, all without interfering with endogenous signalling. In order to use this system to investigate how neuropeptide diffusion regulation, we will control when and where PDF-1 will be secreted from. As PDF-1 diffuses through the worm, it will generate a distinct activation pattern which will set the foundation for an in-depth investigation to understand neuropeptide spatial range of action.

93. Visualization of neuropeptide release sites using nanobodies in C. elegans

Dunkel, Eva; Aoki, Ichiro; Gottschalk, Alexander

It remains elusive whether neuropeptides are released uniformly along neurites or more locally and whether multiple species of neuropeptides expressed in the same neuron are released together or differentially. To clarify these questions, we aimed to visualize the release of neuropeptides near the release sites by capturing them using a nanobody against a fluorescent protein that is co-released with the neuropeptides.
We focused on the AVK neuron since it expresses at least two neuropeptides: FLP-1 and NLP-49 (PMID: 9733518, 30201834 and 30392795). Since FLP-1 and NLP-49 have different effects on locomotion (PMID: 30201834 and 30392795), we hypothesized that the release of these peptides is differentially regulated. To monitor the release of those neuropeptides, we expressed FLP-1 or NLP-49 fused with mCherry in AVK and a nanobody against mCherry on the extracellular surface of body wall muscles or cholinergic neurons. A nanobody is a single-domain antibody (PMID: 25362362); previously, it was fused to a native hypodermal protein to monitor peptide release from dendrites of PVD nociceptors (PMID: 31735664). Expression and recruitment of the nanobody to the plasma membrane in body wall muscles was monitored by intracellularly fused GFP. mCherry released with FLP-1 was seen on body wall muscles when the nanobody construct was expressed there but was absent in a control missing the nanobody sequence, indicating that the nanobody successfully captured mCherry. The mCherry signal was predominantly distributed in the anterior region of C. elegans. Expression of the nanobody construct in cholinergic neurons, which overlap the AVK process, resulted in mCherry capturing along the entire domains of cholinergic neurons.
Taken together, we developed a tool that can monitor the release of any neuropeptide of interest on any cells of interest. We are currently analyzing the distribution of mCherry released with NLP-49 to examine whether FLP-1 and NLP-49 are released from different sites.

94. A master neuron with depolarized steady membrane potential controls overall locomotor states

Meng, Jun; Ahamed, Tosif; Yu, Bin; Hung, Wesley; El Mouridi, Sonia; Wen, Quan; Gao, Shangbang; Zhen, Mei; Boulin, Thomas; Leclercq-Blondel, Alice; Gendrel, Marie; Wang, Zenan; Chen, Lili

Mutually exclusive motor behaviors are often assumed to be driven by circuits that are mutually inhibitory. In the nematode C. elegans, motor circuits that propel the animal's body movement during forward and backward locomotion are gated by the premotor interneurons AVB and AVA, respectively. Previous research has shown that AVA and AVB exhibit anticorrelated activity and may be mutually inhibitory. In this study, we confirm previous findings that AVA holds a very depolarized steady membrane potential. By perturbing AVA's

endogenous potassium and sodium leak-current equilibrium, we show that increased AVA activity potentiates both forward and backward movement. Conversely, decreased AVA activity results in inhibited forward and backward movement. Measuring AVA and AVB neuronal activity during freely moving animals reveals that AVA functions at a two- time scale to regulate the overall motor states. These findings contradict the consensus model in the literature whereby AVA drives backward locomotion only. We present optogenetic stimulation protocols that resolve this contradiction: phasic activation of AVA yields backward locomotion, while tonic activation potentiates forward locomotion. We show that Tonic release of acetylcholine from AVA even when it is at “rest” is essential for AVA to regulate forward locomotion by stimulating AVB. AVA therefore functions as a master neuron that determines the animal's locomotion state.
These results demonstrate that a single neuron can regulate holistic motor circuits to generate two mutually exclusive behaviors.

95. **A sol-gel maturation of MEC-2/Stomatin governs mechanotransduction during touch**
Sanfeliu-Cerdán, Neus; Mateos, Borja; Garcia-Cabau, Carla; Català-Castro, Frederic; Porta-de-la-Riva, Montserrat; Salvatella, Xavier; Krieg, Michael; Ribera, Maria

The transduction of mechanical stresses such as tension or compression is critical for survival and quality of life. Whereas most ion channels and receptors behind mechanotransduction have been identified, it is still uncertain how forces are transmitted to the molecular mechanosensors. Here, we show a novel mechanism by which an essential and highly conserved protein for touch sensation in C. elegans – MEC-2 stomatin – undergoes a rigidity transition during maturation from a liquid and mobile pool to force-sensing, solid and immobile condensates in touch receptor neurons. In vitro biochemistry assays with purified proteins revealed that MEC-2 reconstitutes into largely deformable liquid droplets. Using optical tweezers measurements in vitro and diffusion assays in vivo, we observed that MEC-2 matures into gel-like platforms, with a strong frequency dependent viscoelastic property, suggesting that it can transmit forces on short (>1Hz) but not on long timescales. We found that this maturation depends on an unstructured proline-rich motif at the C-terminus of MEC-2, which has SH3 binding properties. By a combination of neuronal calcium imaging and behavioural assays, we showed that UNC-89 – a titin/obscurin homolog – interferes in touch and its SH3 domain co-condensates with MEC-2 mature pools in mechanosensitive platforms. Together, our data suggest that MEC-2 maturation tunes its function from proper transport along neurites to a filter for mechanical stresses, as well as uncovers, for the first time, an unexpected role of UNC-89 in neurons. Our work motivates future studies towards a general mechanism behind mechanosensation.

96. **Advanced microfluidic assays for the study of C. elegans**
Karimi, Shadi; Krieg, Michael; Sanfeliu-Cerdn, Neus

Mechanical stress is a considerable risk factor in the etiology of neurodegenerative disorders, thus, it is essential to uncover the molecular pathway that protects neurons against chronic mechanical insults. Which signalling proteins are activated and how the cytoskeleton protects neurons from large deformations under constant mechanical stress is currently little explored.

At NMSB lab, we study the subtle, intermediate damages to a neuron through dynamic mechanically active microfluidic platforms. To mimic the repetitive deformation of mechanosensory cells that are subjected to cyclic stresses as a natural part of their function, we designed two different devices for both in vitro and in vivo applications.
Firstly, we fabricated a cell stretcher (ExCell) that can mechanically stimulate cells growing in culture by stretching and compressing them. This occurs via adhering cells to the treated PDMS cell culture dish and subjecting them to long-term mechanical strain for live cell imaging. ExCell is adaptable to both upright and inverted microscopes and integrated with a built portable top-stage incubator that allows regulation of temperature, humidity and CO2 level. We used ExCell to apply long-term stress to C. elegan cells for multiple durations and compared the results with the control dish by RNA sequancing.
Next, we created a worm compressor that operates with a programmed pressure pump, enabling us to apply mechanical stimulus repetitively to the whole organism in high-throughput and observe the cell responses in real-time. The correlation between the findings of these two platforms leads to a thorough investigation of the mechanobiology of tissues, which were impossible to obtain with traditional cell culture methods. Ultimately we can target specific pathways associated with mechanical stresses and will provide novel insights into our understanding of the accumulation of damage product secondary to mechanical stress, aggregated tau proteins, depolymerized cytoskeleton, etc.

97. **Age-related neuronal changes, lifespan pathways and maintenance of neuronal architecture**

CHABI, Yann; KHANDEKAR, Anagha; LIU, Ju-Ling; BENARD, Claire; CHABI, Yann

Cognitive decline during aging is well-known in humans, however the mechanisms by which nervous system dysfunction is triggered during aging are poorly understood. Previous studies in *C. elegans* reported age-related morphological changes of neurons (including by Pan*et al* , 2011; Tank *et al* , 2011; Toth *et al* , 2012). We extended these analyses by systematically surveying age-related neuronal changes in wild-type animals and found that neuron-type specific structural alterations occur across the entire nervous system during normal aging. We also found that age-related neuronal changes can be uncoupled from lifespan extension, as neuronal morphological alterations are not robustly delayed in all the long-lived mutants examined, consistent with findings on healthspan analysis (Bansal *et al* , 2015). One exception, however, is that of calorically restricted animals : using the genetic model of the *eat-2* mutants (ad465 and *ad1116*), we find that most age-related neuronal alterations are delayed compared to wild-type animals. To dissect the molecular pathways responsible for delaying age-related neuronal alterations, we have first determined that the transcription factor *pha-4* /FOXA, necessary for the longevity of *eat-2* mutants, is also required for the neuronal protective effects of dietary restriction. We are exploring the interplay between caloric restriction and neuronal maintenance molecules, and we find that the loss or gain of function of*sax-7* affects neuronal structures in distinct ways that inform us on the process of neuronal aging. SAX-7 is homologous to the L1CAM family of cell adhesion molecules in mammals, where it functions to preserve cognitive abilities in adults. Our findings indicate that the interaction between molecular mechanisms dedicated to the lifelong maintenance of neuronal architecture and lifespan determination are key to age-related neuronal change. Given the conservation between the human and *C. elegans* genomes, and in neuronal processes, the genes that protect from or promote neuronal decline in

C. elegans will advance our knowledge of the principles underlying neuronal maintenance and aging and may provide insights into age-related neurodegenerative diseases.

98. **AMsh uptake of ciliary-derived Extracellular Vesicles challange glial proteostasis**
Razzauti Sanfeliu, Adria

The amphid sensory system is composed of 2 glial cells forming the amphid channel and 12 ciliated sensory neurons. We recently observed that Extracellular Vesicles (EVs) are released by outward budding (ectocytosis) from a subset of amphid sensory cilia (Razzauti and Laurent, 2021). EVs produced from the cilium tip are released to the environment. EVs produced from the cilium base are internalized by the surrounding AMsh glia. Defects in IntraFlagellar Transport (IFT) lead to cargo accumulation that can occur at the ciliary tip and/or the ciliary base. Consequently, more EVs are released from the cilium tip or from the cilium base as a safeguard mechanism to maintain cilia homeostasis. Previous observations showed the amphid channel size is set by AMsh secretions and modified in some (but not all) IFT mutants, suggesting a communication between the sensory cilia and AMsh glia. We checked whether increased export of EVs to AMsh glia might affect AMsh. We observed that in mutants where EV export to AMsh is enhanced also display an increased AMsh size and length. This is often accompanied by blebbing at AMsh posterior end. In severe cases, release of AMsh cell content occurs by cellular fragmentation or exophere production. Interestingly, we also observed abnormal AMsh morphology when we overexpressing aggregating proteins. Our current results suggest that increased ciliary EV export to AMsh might disturb AMsh protein homeostasis. We characterize this phenomenon.

99. **An imaging pipeline for behavioral and neural dynamics of pharyngeal motion patterns**
Zhang, Hongruo; Xu, Alice Linyan; Gao, Rory; Torkashvand, Mahdi; Ahamed, Tosif; Venkatachalam, Vivek; Zhen, Mei

C. elegans performs two relatively independent but coordinated motion patterns by its food ingestion organ, the pharynx. This provides a good model to study the circuit basis of motion patterns coordination. However, it is still unclear how the two motion patterns are coordinated by the pharyngeal neuromuscular system. Here, we developed a fluorescent bacteria-based imaging and signal extraction pipeline that can monitor the contraction of all pharyngeal muscles in 1). milliseconds time resolution and 2). calcium imaging compatible manner. Long-term coordinated pharyngeal motion patterns can be maintained under a less-retrained imaging condition, using a mutant strain with deficient body locomotion but no deficiency in feeding motion patterns. When fed with mCherry-expressing E. coli, the detailed timing of the contraction of all pharyngeal muscles can be robustly measured through the intensity change of fluorescent signal within the pharyngeal lumen. We have developed an automatic segmentation pipeline to extract the lumen fluorescent signal from the recordings. Further, the muscle and neuron activity can be monitored using the calcium indicator at the same time when characterizing the feeding motion patterns in this imaging setup. This allows us to study the correlation between pharyngeal motion patterns and the activity of the pharyngeal neuromuscular system. We aimed to determine the circuit and cellular components within the neuromuscular system that participate in the coordination of the motion patterns using this imaging pipeline.

100. **Asymmetry and sexual dimorphism of the adult PVD neurons**

Iosilevskii, Yael; Podbilewicz, Benjamin

The C. elegans bilateral PVD neuron pair develop a complex yet stereotypical dendritic branching pattern. This ordered structure is maintained in early adulthood, yet gradually accumulates additional processes and becomes disorganized. Little is known regarding the bilateral differences in this homeostatic process. We show that early adult animals display robust dorso-ventral and left-right asymmetries for additional branching presence.
Using mutant analysis, we demonstrate that some asymmetries do not depend on the initial degree of hyperbranching. Further, these asymmetries do not reflect enhanced plasticity, as determined by a similar outgrowth response to dendritic damage. Additionally, we show that male PVD neuron structure retains some asymmetries seen in the hermaphrodite, but presents additional, sexually dimorphic, traits.

101. **B-Raf/lin-45 is a signaling hub for genes involved in neurodegeneration**

Cieri, Federica; Santonicola, Pamela; Zampi, Giuseppina; Hensel, Niko; Tapken, Ines; Detering, Nora Tula; Claus, Peter; Di Schiavi, Elia

The B-Raf orthologue *lin-45* belongs to MAPK/ERK pathway, is involved in the regulation of cellular proliferation and differentiation and shows a Ras GTPase binding activity. Beside its well-known role in vulva formation, *lin-45* is also expressed in neurons, including motoneurons, and is critical for chemo- and thermo-sensory behaviors and locomotion. Using a phospho-array approach on Spinal Muscular Atrophy (SMA) mice and network-biology, we identified 38 dysregulated proteins belonging to different pathways involved in motoneuron degeneration with B-Raf as its signaling hub. In SMA, motoneurons degenerate because of mutations in the Survival Motor Neuron 1 (*SMN1*) gene. We investigated in *C. elegans* the role of B-Raf/*lin-45* in degenerating motoneurons (MNs) observing that *lin-45* expression is reduced in pre-symptomatic *smn-1(KO)* animals. We rescued the neurodegeneration and locomotion defects caused by *smn-1* silencing in D-type MNs by re-expressing *lin-45* specifically in motoneurons*,* demonstrating that *lin-45* can play a cell-autonomous neuroprotective role when *smn-1* is down-regulated. Moreover, a *lin-45* hyperactive isoform enhances the rescue effects. The neurodegeneration caused by *smn-1* silencing increases with animal ageing. Using inducible transgenics, we over- expressed *lin-45* from L2 stage, when the degeneration has already started and 19 D-type motoneurons are generated, demonstrating that*lin-45* protects from motoneuron loss post-symptomatically rather than interfering with neurogenesis. Finally, using genetic and pharmacology approaches, we demonstrated that *lin-45* rescue occurs through the MAPK/ERK pathway. These data were confirmed in mammalian setting, supporting a B-RAF role in neurodegeneration and in the *Smn1* pathway. To elucidate the role played by other dysregulated proteins identified in the phospho-array experiment, we tested their *C. elegans* homologs to identify the possible modifier genes of *smn-1* induced neurodegeneration in D-type MNs. We identified 9 suppressor and 9 enhancer genes among all the 38 genes tested. Using cell-specific RNAi, we demonstrated that 11 play a role in neurons and in particular, 3 of those specifically in D-type MNs. These data unveil a neuroprotective role of a network of genes in MNs which are candidate targets for future therapies in SMA.

102. **Biomonitoring of microbial and chemical indoor air toxins with C. elegans nematodes**
Koskinen, Päivi J; Paavanen-Huhtala, Sari; Kalichamy, Karunambigai S; Pessi, Anna-Mari; Häkkilä, Sirkku; Saarto, Annika; Andersson, Maria

The *Caenorhabditis elegans* nematodes have been found to be suitable for biomonitoring of multiple types of environmental agents, as their chemosensory neurons can efficiently detect volatile odorants or soluble flavors. Thereby the animals can discriminate between beneficial and harmful substances in their living environment, find food and avoid pathogens, many of which are hazardous also for humans. This has prompted us to test the possibility that *C. elegans* could be used also to monitor indoor air quality.
Indoor air problems are far too common in schools and other public buildings, where they weaken the health, well-being and working capacity of exposed people. The current methods to evaluate indoor air health hazards are limited and mainly based on cellular assays. However, multicellular organisms, such as *C. elegans* may provide additional advantages to predict their effects on human tissues. Therefore, we have used transgenic*C. elegans* strains carrying stress-responsive fluorescent reporters, and evaluated their abilities to sense microbial or chemical toxins, especially those that are present in moisture-damaged buildings. In animals exposed to such agents, we have reproducibly observed time- and dose-dependent fluorescent responses, which could be quantitated by either microscopy or spectrometry. Furthermore, our results have correlated well with those obtained from cell-based assays. Thus, the *C. elegans* nematodes may offer an easy and comprehensive method to monitor overall indoor air toxicity, but further studies are needed to validate their ability to distinguish between healthy and harmful buildings also under field conditions.

103. **Body Mechanics Facilitates Contact-mediated Mate Recognition in C. elegans**
WENG, JEN-WEI

Physical contact is prevalent in the animal kingdom to recognize suitable mates by decoding the information of sexes, species, and maturity. Although chemical cues for mate recognition are extensively studied, the role of mechanical cues in mate recognition remains elusive. Here we show that C. elegans males recognize conspecific and reproductive mates through physical contact, and the attractiveness of potential mates is dependent on sex and developmental stages of the hypodermis. In hypodermis, a group of evolutionarily conserved cuticular collagens, dpy-2, dpy-7, and dpy-10, is required and sufficient to promote mate attractiveness by assembling adult- and hermaphrodite-specific cuticles. We find that collagens control mate attractiveness via maintaining body mechanics but not surface features of cuticles, and the decrease in mate attractiveness is associated with low mating efficiency. We further demonstrate that body mechanics serves as a permissive cue to facilitate mate recognition evoked by yet unidentified surface associated signal. Our study thus extends the repertoire of sensory cues for mate recognition in C. elegans, and provides a paradigm to study sensory decoding of social information.
keywords : C. elegans, mate recognition, physical contact, body mechanics

104. Building the presynapse: How SYD-2, HLB-1 and CLA-1 work together in the formation of a functional presynaptic ultrastructure

Cockram, Lewis; Kittelmann, Maike

Neurons are unique in their capacity to build complex systems of information transfer through their ability to communicate with one another via synapses. Chemical synapses accomplish this through the release of neurotransmitter from a presynaptic neuron which then stimulate a target cell. Coordination of these signals requires fine spatiotemporal control through the presynaptic active zone scaffold that is visible under electron microscopy as “dense projection”. While several of its component proteins are known, their individual removal typically has minimal effects on dense projection structure in C. elegans, suggesting a degree of inherent redundancy. This presents a challenge for deducing the function of these proteins in active zone scaffold formation, and consequently the role of the structure in neurotransmission. To overcome this, we have generated double and triple mutant strains of active zone proteins SYD-2, CLA-1 and HLB-1 and assessed locomotor behaviour, neurotransmission and synaptic component localisation to understand the effects of these combinatorial mutants at both the whole animal and single synapse level. We demonstrate that SYD-2 loss causes the single greatest reduction in both locomotor activity and synapse integrity with little additional influence of HLB-1 and CLA-1. However, CLA-1 and HLB-1 may contribute to cholinergic neurotransmission independently. These findings confirm the status of SYD-2 as the primary presynaptic organiser.
Further investigations examining synaptic ultrastructure using electron microscopy will clarify the collective contributions of these proteins to active zone scaffold ultrastructure and synaptic vesicle recruitment.

105. Calpain mediated proteolysis of β-spectrin accelerates proprioceptive decline in C. elegans

Lin, Li-Chun; Das, Ravi; Malaiwong, Nawaphat; Krieg, Michael; Scholz, Nicole; Dahse, Anne-Kristin

Proprioceptive feedback provides a neural representation of body mechanics to the CNS, which allows the formation of repetitive locomotion pattern in many animals, including humans. In *Caenorhabditis* (*C.*) *elegans* aging manifests as a decline in the animals’ anatomical and functional features, such as tissue integrity, motility and their repetitive gait cycle. Many, including us, have observed structural changes and functional deterioration of the proprioceptive neurons in aging animals. At the cellular level, one of the primary age-dependent changes include protein aggregation, in part due to a reduced cellular unfolded protein response of the ER. While it is known that one of the key cytoskeletal components, spectrin, plays a crucial role in protecting axons and dendrites from mechanical stress, it is less known about its regulation and role during aging. Here, we observed that aged animals crawl with subtle, but significantly larger body bends, indicative for a defect in proprioception, reminiscent of animals defective in DVA-specific spectrin network (Das, Science Advances, 2021). This defect correlated with reduced curvature-correlated calcium activity of DVA and an overall reduced mechanical tension in the spectrin network of old animals. Using classical protein biochemistry, we observed that UNC-70/β-spectrin gets cleaved in aged animals, and pulldown experiments suggest that UNC-70 interacts with cytoplasmic proteases and heat-shock proteins. We thus hypothesize that substrate-level regulation of spectrin cleavage

through CLP-1, a calcium-sensitive cysteine protease that is overrepresented in DVA proprioceptors, leads to proprioceptive defects in early age. Indeed, upon conditional knockout of*clp-1* in a single proprioceptive neuron, DVA, we observed a change in the body bending amplitude. Our study reveals novel insights on age-associated molecular changes, which not only provides valuable information on potential biomarkers for aging but also builds ground for the identification of potential therapeutics to treat age related neurodegenerative diseases.

106. CED-3/caspase and opposing kinesins direct mitochondrial confinement to govern Compartmentalized Cell Elimination

Sharmin, Rashna; Carmona, Sara; Shaham, Shai; Clark, Ginger; Juanez, Karen; Elkhalil, Aladin; Pellegrino, Mark; Ghose, Piya

Programmed cell death is essential for normal development and homeostasis, particularly in the nervous system. Morphologically complex cells are defined by their elaborate processes, such as axons and dendrites in neurons. While such complex cells are very common, the mechanism behind their programmed elimination remains elusive, as is how they are eliminated under pathological conditions or following injury. We discovered a novel 'tripartite' killing program that dismantles the sex-specific CEM neurons and the morphologically complex tail-spike epithelial cell (TSC) during*C. elegans* embryonic development. This program, we term Compartmentalized Cell Elimination, or CCE, is distinguished by three cell regions dying in three different ways. Remarkably, the single process/dendrite of these cells shows two disparate elimination morphologies in two subcellular domains, morphologically similar to different types of developmental pruning, retraction and fragmentation, as well as injury-induced Wallerian degeneration and pathological neurodegeneration. We present CCE as a powerful *in vivo* model for region-specific cell elimination. We report that mitochondrial retrograde transport, mediated by the Kinesin-1 homolog UNC-116, and soma sequestration of mitochondria are a pre-requisite for CCE. Our genetic evidence suggests that soma confinement of mitochondria is accomplished by the targeting of the anterograde motor UNC-104/Kinesin-3 by CED- 3/Caspase. Our findings present a regulatory mechanism for the novel phenomenon of CCE, introduce a novel,*in vivo* , caspase target and provide insight into regulation of pruning/localized cell elimination.

107. Combinatorial effects of bacterial co-culture engages distinct sensory signaling pathways in C. elegans to modulate behavior

O'Donnell, Michael; Dasgupta, Madhumanti; Jackson III, Andrew

Caenorhabditis elegans uses its highly-developed chemosensory system navigate through its microbe-rich natural environment. Gut microbes can also alter sensory-driven behavior of *C. elegans* by producing neuroactive chemicals. *Providencia alcalifaciens* , a beneficial gut bacterium, produces tyramine, which is converted to octopamine in *C. elegans,* and this results in reduced odor aversion. Here we show that *C. elegans* feeding on a monoculture of non-pathogenic *Pseudomonas* species isolated from worms show decreased aversive responses to octanol, a volatile repellent, compared to feeding on the laboratory food, *E. coli* . This decreased octanol aversion occurs via modulation of serotonergic signaling – a pathway independent of the tyraminergic pathway we previously described in *Providencia* .

Interestingly, animals feeding on a co-culture of *Providencia* and *Pseudomonas,* show unique aversive responses to octanol: these animals exhibit more rapid response than animals grown on monocultures of either strain or to the *E. coli* control condition. This synergistic effect of co-culture also involves serotonin receptors, SER-5 and SER-7, suggesting that novel metabolites are likely produced cooperatively by these bacteria. Additionally, we find that animals feeding on co-culture results in synergistic increases in the population of both bacteria inside the gut. Metabolomic analysis of animals feeding in these conditions show reduction in levels of a recently discovered class of putative signaling molecules called octopamine modular glucosides, compared to animals fed on*Providencia* . These results suggest that bacteria-bacteria metabolic interactions can prevent chemosensory modulation by specific gut microbes, while also resulting in novel host behavioral phenotypes.

108. Connecting few to many: decoding FLP-receptor interactions in experience-dependent plasticity

De Fruyt, Nathan; Zels, Sven; Vandewyer, Elke; Ripoll Sánchez, Lidia; R. Schafer, William; E. Vértes, Petra; Beets, Isabel

Neuropeptides are important modulators of neural circuits and behaviors, which mainly act through extrasynaptic signaling via G protein-coupled receptors (GPCRs). In most animals more than a hundred neuropeptide-encoding genes have been identified, many of which are evolutionarily ancient and highly conserved. Yet, our understanding of the functional organization of this dense peptidergic signaling network is limited. Using reverse pharmacology, we have mapped the biochemical neuropeptide signaling network in C. elegans and identified over 450 neuropeptide-receptor interactions. Bipartite network analysis revealed different modules and topologies for the signaling networks of the two main families of peptide-GPCR ligands, the RFamide peptides (FLPs) and the neuropeptide-like proteins (NLPs). We find that NLPs signal with high specificity, typically activating a limited number of GPCRs. By contrast, the FLP signaling network shows a high level of crosstalk with ‘many to one’ and ‘one to many’ receptor interactions. Using reverse genetics, we are investigating the functions of these complex neuropeptide-receptor interactions in the modulation of neural circuits and behavior. We find that FLPs signaling to multiple specific neuropeptide receptors, such as FLP-8 and FLP-14, are among the most abundant and highly conserved peptides in nematodes and are involved in the experience-dependent modulation of multiple sensory circuits, including gustatory aversive learning and the plasticity of oxygen avoidance. We are currently dissecting the cellular network underlying these functions. This will provide insight into the functional organization of neuropeptide signaling networks that mediate experience-dependent plasticity.

109. Correcting motion induced fluorescence artifacts in two-channel neural imaging

Creamer, Matthew; Chen, Kevin; Pillow, Jonathan; Leifer, Andrew

Imaging neural activity in a behaving C. elegans presents unique challenges because motion from an animal's movement creates artifacts in fluorescence intensity time-series that are difficult to distinguish from neural signals of interest. One approach to mitigating these artifacts is to image two channels simultaneously; one that captures an activity-dependent fluorophore, such as GCaMP, and another that captures an activity-independent fluorophore

such as RFP. Because the activity-independent channel contains the same motion artifacts as the activity-dependent channel, but no neural signals, the two together can be used to identify and remove the artifacts. Existing approaches for this correction, such as taking the ratio of the two channels, do not account for channel independent noise in the measured fluorescence. Moreover, no systematic comparison has been made of existing approaches that use two-channel signals. Here, we present Two-channel Motion Artifact Correction (TMAC), a method which seeks to remove artifacts by specifying a generative model of the fluorescence of the two channels as a function of motion artifact, neural activity, and noise. We use Bayesian inference to infer latent neural activity under this model, thus eliminating the motion artifact present in the measured fluorescence traces. We further present a novel method for evaluating ground-truth performance of motion correction algorithms by comparing the decodability of behavior from neural recordings in two types of behaving worms; a recording that had both an activity-dependent fluorophore (GCaMP and RFP) and a recording where both fluorophores were activity-independent (GFP and RFP). A successful motion-correction method should decode behavior from the first type of recording, but not the second. We use this metric to systematically compare five models for removing motion artifacts from fluorescent time traces. We decode locomotion from a GCaMP expressing animal 15x more accurately on average than from control when using TMAC inferred activity and outperform all other methods of motion correction tested.

110. **Decoding the role of the NFY complex in nervous system development.**
Moreira, Pedro; Papatheodorou, Paul; Deng, June; Cornell, Rebecca; Pocock, Roger

The establishment of correct nervous system architecture during development is an exceptionally complex process requiring precisely controlled gene expression. However, the transcriptional networks responsible for correct neuronal specific and pan-neuronal gene expression is still poorly understood. The Nuclear Factor Y (NFY) trimeric complex is one of the most conserved transcription factors in eukaryotes and it comprises the NFY-A, - B and -C subunits that regulate gene expression through binding specific regulatory motifs in the genome.
In an unbiased forward genetic screen, we identified the NFY complex as a key regulator of neuronal fate acquisition and pan-neuronal gene expression. Our data show that the NFY regulates PVQ neuronal fate acquisition, by regulating neuron-specific gene batteries. Moreover, we find that the NFY complex regulates neuronal function by regulating the expression of pan-neuronal genes including rab-3, snb-1 and unc-11. By performing direct site mutagenesis, we confirmed that the NFY directly regulates PVQ specific gene batteries and pan-neuronal gene expression in different neuronal sub- types.
In conclusion, in this work we describe a novel function of the NFY complex in controlling neuron-specific and pan-neuronal gene expression.

111. **Developmental history modulates adult olfactory behavioral preferences via regulation of chemoreceptor expression in C. elegans**
Rogers, Travis

Developmental experiences play critical roles in shaping adult physiology and behavior. We and others previously showed that adult C. elegans which transiently experienced dauer arrest during development (PD: post-dauer) exhibit distinct gene expression profiles as compared to control adults which bypassed the dauer stage. In particular, the expression patterns of subsets of chemoreceptor genes are markedly altered in PD adults. Whether altered chemoreceptor levels drive plasticity in chemosensory behaviors in PD adults is unknown. Here we show that PD adults exhibit enhanced attraction to a panel of food-related attractive volatile odorants including the bacterially produced chemical diacetyl but do not alter their responses to volatile repellents. Diacetyl-evoked responses in the AWA olfactory neuron pair are increased in both dauer larvae and PD adults, and we find that these increased responses are mediated in part via upregulation of the diacetyl receptor ODR-10 in AWA likely via both transcriptional and post- transcriptional mechanisms. We report that transcriptional upregulation of odr-10 expression in dauer larvae is in part mediated by the DAF-16 FOXO transcription factor. Via transcriptional profiling of sorted populations of AWA neurons from control and PD adults, we further show that the expression of a subset of additional chemoreceptor genes in AWA is regulated similarly to odr-10 in PD animals. Our results suggest that developmental experiences may be encoded at the level of olfactory receptor regulation, and provide an elegant mechanism by which C. elegans is able to precisely modulate its behavioral preferences as a function of its current and past experiences.

112. Differentiated dynamic response in C. elegans chemosensory cilia

Bruggeman, Christine; Haasnoot, Guus; van Krugten, Jaap; Danne, Noemie; Peterman, Erwin

Cilia are membrane-enveloped organelles that protrude from the surface of most eurokaryotic cells and play crucial roles in sensing the external environment. For maintenance and function, cilia are dependent on intraflagellar transport (IFT). Here we investigate the response of the Caenorhabditis elegans phasmid chemosensory cilia to chemical stimuli to better understand the process of chemosensation. To do so we use a combination of microfluidics, to apply stimuli with a high degree of temporal control, and fluorescence microscopy, to visualize structure and dynamics of the cilia, as well as the neuronal response. We found that chemical stimulation resulted in unexpected changes in IFT and ciliary structure. Notably, stimulation with hyperosmotic solutions or chemical repellents resulted in different responses, not only in IFT, ciliary structure and cargo distribution, but also in neuronal activity. We found that in response to chemical repellents, such as SDS or Cu2+, the ciliary axoneme shrinks and IFT components are redistributed towards the ciliary base. In response to hyperosmotic stimuli, IFT components accumulate at the ciliary tip due to inhibition of retrograde transport. Currently we are trying to unravel how IFT and neuronal activity are precisely linked on the molecular level. Repetitive stimulation with chemical repellents results in lower neuronal activity every subsequent exposure, pointing towards a mechanism of desensitization. This suggests that IFT plays a role in regulating the chemosensory response. Taken together, our findings show that cilia are able to sense and respond to different external cues in distinct ways, highlighting the flexible nature of cilia as sensing hubs.

113. Distinct regeneration manners of head ganglia neurons in C. elegans

Tsukada, Yuki; Mori, Ikue

Neuronal regeneration varies depending on neuronal types and positions. This variety of regeneration builds up robust resilience of neural circuits by providing appropriate recovery for a specific circumstance. While the regeneration of touch neurons and motor neurons located at the ventral nerve code or lateral body area are actively studied, there are few reports about the regeneration of head ganglia neurons (Byrne and Hammarlund 2017). Previous reports about laser surgery of head neurons described no regeneration of head neuronal axons (Gabel et al. 2008). Here, we report distinct regeneration manners of AFD and AWC sensory neurons in head ganglia after femtosecond laser surgery. We found that the thermo-sensory neuron AFD shows a variety of regeneration manners after femtosecond laser surgery of the axon near to the body surface. The regenerating axons showed several manners of extension such as sprouting, branching, and wandering. Although the same axonal positions were cut, sprouting positions also varied such as in axon, soma, and dendrite. After the surgery, most of the regenerating axons extended toward the nerve ring where a former part of its own is located. That implicates the existence of the guidance cues toward the nerve ring during the regeneration. Regenerated axons and even the regenerated neurite sprouting from soma or dendrite directed to the nerve ring and made connections with the rest of the axons of its own at the nerve ring. AWC, which is previously reported to show no regeneration (Gabel et al. 2008), also exhibited regeneration from dendrite to the nerve ring. Thus, neurons in the head lateral ganglion exhibited robust regeneration of axon in adult or L4 stage injury to recover connection from soma to nerve ring. On the other hand, laser surgery of the axon at nerve ring region did not show any regeneration at all. We are exploring molecular mechanisms underpinning the observed head neuronal regeneration and pkc-1 may play a role in inhibitory mechanisms for sprouting neurite in the regeneration of AFD neurons. Our findings may provide a key understanding of both resilience of neural circuits and circuit construction.

114. Evolution of neuronal activity and system entropy in C. elegans during emergence from isoflurane anesthesia

Connor, Christopher; Chang, Andrew; Gabel, Christopher

The anesthetized state is characterized by a suite of behavioral and physiological responses, as well as by characteristic alterations in EEG patterns. However, these measures tell us little about the neuron or circuit-level physiological action of anesthetic agents. Historically, C. elegans has served as a powerful model organism in the study of the basic mechanisms of anesthesia. Here we employ single-neuron calcium imaging in C. elegans to probe the basis of volatile anesthetic action on neuronal function. Performing volumetric fluorescence imaging, we measure neuronal activity across a large portion of the C. elegans nervous system at distinct states of isoflurane anesthesia as well as during dynamic emergence from the anesthetized state. Power spectral density analysis of individual neuron activity demonstrates significant elevations in high frequency activity in the anesthetized animal which gradually subsides during emergence over ~1hr. To gain further insight into neuronal mechanisms of anesthesia and emergence we turned to information theoretic metrics based on how entropy, or information content, is shared in the time-evolution of signals from paired neurons. We find that traditional entropy-derived metrics such as mutual information and transfer entropy do not strongly distinguish between anesthetized and non- anesthetized animals. By contrast, we have developed novel entropic metrics that can strongly differentiate the awake and

anesthetized states and reveal gradual emergence dynamics that resolved over roughly two hours. The slow resolution of these metrics contrasts with the quicker resolution of high frequency activity immediately post-anesthesia, which suggests discrete phases of recovery. Our analysis reveals how information flow is altered during the anesthetized state, how this flow returns to baseline during emergence and provides a novel understanding of the mode of anesthetic action on a complete nervous system.

115. Evolutionarily conserved neuropeptidergic networks mediating sensory cross-modulation in C. elegans

Van Damme, Sara; Beets, Isabel; Geens, Ellen; De Fruyt, Nathan; Vandewyer, Elke; Zels, Sven

To capture the multisensory world, nervous systems rely on sensory circuits that do not work in isolation but are influenced by other senses. Sensory cross-talk allows animals to adjust behavior and decisions based on multisensory input. Given its adaptive value, it is not surprising that sensory cross- modulation is found across metazoans. However, the molecular and cellular mechanisms underlying this experience-dependent plasticity are not well understood. Neuropeptides represent an evolutionarily ancient and diverse group of neuromodulators that are key regulators of sensory circuits. Using a reverse pharmacology approach, we biochemically mapped the neuropeptide-receptor signaling network in*C. elegans* . This revealed a dense network of neuropeptide-receptor pairs that, as in mammals, allows broad potential communication across sensory circuits. To uncover functions of these peptidergic connections, we are dissecting their effects on the interaction of circuits signaling aversive oxygen (O2) and carbon dioxide (CO2) stimuli, which have been shown to rely on neuropeptide signaling for cross-modulation. Expression analysis revealed that neurons in the O2 and CO2 circuit express several neuropeptide-receptor pairs, including evolutionarily conserved pathways related to vertebrate somatostatin and insect short neuropeptide F signaling. Mutants defective in these pathways are impaired in the modulation of CO2 avoidance by O2 experience. Using cell-specific rescue and imaging approaches, we are further unravelling the peptidergic circuitry underlying these modulatory effects. This will provide deeper insight into the functional organisation of neuropeptide-receptor signaling networks and their role in sensory cross-modulation.

116. FMRFamide-like neuropeptide FLP-2 and pigment dispersing factor-like neuropeptide PDF-1 may modulate salt chemotaxis of C. elegans by mediating food signals

Xie, Yucheng; Yamada, Koji; Adachi, Takeshi; Kunitomo, Hirofumi; Iino, Yuichi

Neuropeptides modulate a variety of biological processes like foraging, reproduction, learning and memory by regulating neuronal functions such as synaptic transmission between neurons. In this research, we screened neuropeptides that potentially act in salt chemotaxis learning of Caenorhabditis elegans, a type of associative learning in which experience of salt concentration and nutrition modulates the behavior. We found that neuropeptide-overexpression strains for 20 out of 36 tested neuropeptide genes showed defects in salt chemotaxis and/or salt chemotaxis learning. Of these, over- expression of an FMRFamide-like neuropeptide FLP-2 caused a reversed salt concentration preference after conditioning with starvation. FLP-2 was known to promote arousal by mutually activating secretion of PDF-1,

another neuropeptide that has been suggested to represent food abundance (Chen D. D., et al. 2016, Luo J., et al. 2021). We found that over-expression of either FLP-2 or PDF-1 results in salt chemotaxis defects, and they act independently. FRPR-18 and PDFR-1, previously characterized receptors for FLP-2 and PDF-1, respectively, were required for salt chemotaxis defects caused by excess neuropeptides. Mutations in flp-2, pdf-1, or their receptors showed normal salt chemotaxis, suggesting that other molecules may be redundantly involved in this process. FLP-2 antagonistically functions with INS-1 insulin in AIA interneuron to promote reproductive growth (Chai C. M., et al. 2021). AIA-specific expression of flp-2 resulted in chemotaxis defects, implying that FLP-2 may mediate food signals or blocks starvation signals in salt chemotaxis learning via AIA.

117. From connectome to function: connectivity features underlying neuronal population dynamics in the nematode C. elegans

Zimmer, Manuel; Uzel, Kerem; Kato, Saul

The wiring architecture of neuronal networks is assumed to be a strong determinant of their dynamical computations. An ongoing effort in neuroscience is therefore to generate comprehensive synapse-resolution connectomes alongside brain-wide activity maps. However, the structure- function relationship i.e., how the anatomical connectome and neuronal dynamics relate to each other on a global scale remains unsolved. The nematode worm C. elegans with a fully mapped connectome and the availability of a large-scale Ca2+-imaging is an ideal model to study these problems. We therefore generated functional connectivity matrices from nervous system wide Ca2+-imaging recordings and systematically compared them with graph features identified in the C. elegans connectome. We found that few local connectivity motifs and mostly other non-local features such as triplet motifs or input similarities can predict functional relationships between neurons. Surprisingly, quantities such as connection strength and total amount of common inputs do not improve these predictions, suggesting that the network's topology is sufficient. We demonstrate that rich-club neurons in the connectome are key to these relevant graph features. Consistently, inhibition of multiple hub neurons specifically disrupts brain-wide correlations but less-so overall activity levels. We propose that the rich-club architecture, and non-local connectivity features such as triplet motifs and input-similarities provide an anatomical substrate for global brain dynamics.

118. Functions of anti-microbial peptides in neural circuits and behaviour

YANG, Xinyi; De Fruyt, Nathan; R. Schafer, William

Infections caused by pathogenic microbiota are believedto alter the behaviours of animals, yet the mechanisms underlying this are not well understood. Recent research in immunology and neurosciencehas shown that the activation of immune cells can stimulate neuron circuits, which will in turn regulate both innate and adaptive immune response. However, it remains unclear how small, infection-related neuropeptides could contribute to the neuro-immune interactions.

C. eleganscontains several structurally-related antimicrobial peptides (AMP) that are induced under pathogenic conditions. We observed that two of these AMPs, nlp-29 and nlp-24, affect C. elegans behaviour in specific ways. nlp-29 knock-out worms survive longer on lawns of the

pathogen S. marcescenscompared to wild type worms, an enhancement we observed to be coupled with altered avoidance behaviour from pathogenic bacterial. In addition, nlp-29 mutants showed a decreased frequency of egg-laying as well as an altered temporal patter of egg-laying when cultured with S. marcescens,compared to when cultured with OP50. This suggests that nlp-29 not only participate in the modulation of worm antimicrobial behaviours, but also could alter a broader range of worm behaviours under pathogenic conditions.
The G protein-coupled receptor NPR-12 has been reported to act in neurons as the receptor of NLP-29 peptides (Lezi E et al.2017). We observed similar lifespan, avoidance behaviour and egg-laying phenotypes in npr-12 mutants as in nlp-29 mutants. We also observed that NLP-29 peptides can activate NPR-12 expressed in Xenopus oocyte and determined that this receptor signals through theGi/o pathway.
Our next step is to study the specific neurons in which nlp-29 and its receptor mainly function. We also aim at discovering the down-stream signalling pathways of this AMP-related anti-pathogen behaviour regulation and the possible up-stream immunity pathways that triggers nlp-29 function during infection.
We also observed from the salt chemotaxis learning experiment that nlp-24 knock-out worms showed significantly learning defect as compared to wildtype. This intriguing result implicates that AMPs could affect more worm behaviours even when worms are not under pathogenic conditions. We are now focusing on deorphanizing the receptor for NLP-24 and specifying the neurons where NLP-24 modulates the learning behaviour.

119. Genome-wide association study reveals a novel regulator for the nematode nictation behavior

Yang, Heeseung; Andersen, Erik C.; Lee, Daehan; E. Cook, Daniel; Paik, Young-ki; Lee, Junho; Kim, Heekyeong

Dispersal is crucial for many organisms in nature as they can encounter limited resources in a restricted small area. Nictation, a dauer-specific standing behavior, can facilitate phoretic interaction with other organisms and help dauer disperse efficiently. C. elegans is most commonly found as dauer in the natural habitat as nematodes often encounter harsh conditions, indicating that the dispersal and survival of dauers are important in the nematode life cycle. Nictation rates vary between C. elegans wild isolates, which may represent various evolutionary histories. In this study, we performed genome-wide association (GWA) mapping on the nictation of 137 C. elegans wild isolates and further genetic tests to identify a novel regulator of nictation behavior. We discovered a quantitative trait locus (QTL) for nictation behavior on chromosome II using GWA mapping. Moreover, we confirmed that the nta-1 gene in the QTL regulates nictation behavior. nta-1 contains many natural variants in its sequence and is predicted to have oxidoreductase activity. The variants in the promoter region induce expression in different tissues, implying that spatial regulation of nta-1 expression underlies natural variation in nictation.

120. Global brain dynamics during food search behaviour

Rey, Ulises; Fieseler, Charles; Hille, Lukas; Zimmer, Manuel

Classical and modern ethology studies suggest that animal behavior consists of discrete behavioral states and motor actions, which are organized hierarchically. The nature of

transitions between behaviors has been described at the behavioural level, but how these transitions are implemented at the level of global brain dynamics is less understood. Having access to whole brain single neuron recordings would allow us to improve our understanding of how animals organize these behavioral transitions at the mechanistic level. For that purpose, we 1) built a custom confocal microscope microscope which allows us to perform whole brain activity recordings with single neuron resolutions in freely behaving animals, 2) we designed an experimental assay where worms perform ethologically relevant behavioural transitions and 3) we developed a machine learning based analysis pipeline to extract single neuron traces from the confocal datasets (see Charles Fieseler poster). In particular, we want to investigate how the worm switches between different food searching strategies (e.g. local search - global search) by simultaneously analyzing its behaviour and its global brain dynamics. With this holistic approach we aim to understand better the underlying neuronal mechanisms that produce the different navigation strategies

121. Gut-brain sphingolipid signaling in learned microbial aversion induced by mitochondrial stress in C. elegans

Pan, Chun Liang; **Wu, Yu Chun**

Animal behaviors are shaped by internal physiological states, but signals that arise in the peripheral tissues to modulate behavioral plasticity are incompletely understood. Disruption of internal physiological functions, including mitochondrial inhibition, triggers bacterial avoidance behavior in*C. elegans* that displays key features of aversive associated memory. How injury-associated signals derived from non-neural tissues promote learned aversive behavior under mitochondrial insults in *C. elegans* remains unclear. We found that *sphk-1* , which encodes a sphingosine kinase, was required for bacterial avoidance under mitochondrial insults, and it functioned in the intestine and hypodermis to regulate this aversive associated memory.
Pharmacological experiments indicated that sphingosine-1-phosphate (S1P) produced by SPHK-1 was a key signal for learned aversion induced by mitochondrial insults. Through gene expression profiling by RNA-sequencing, we identified *lpr-3* , which encodes a lipocalin lipoprotein, as required for learned aversion. Genetic analysis indicated that *lpr-3* and *sphk-1* functioned in the same pathway. Furthermore, we found the GPCR gene *C24B5.1* regulated learned aversion and functioned in RIC, a key modulatory neuron for aversive memory. These findings make C24B5.1 a putative S1P receptor in *C. elegans* , the identity of which remains obscure so far. Our calcium imaging experiments further indicated that S1P and C245B.1 were required for the activity of RIC neurons induced by mitochondrial insults. Together these observations provide mechanistic insights into gut-brain metabolic signaling that enables aversive learning and memory formation under mitochondrial insults.
(supported by the Ministry of Science and Technology, MOST 109-2320-B-002-019-MY3, MOST 110-2320-B-002-057-MY3)

122. High-fidelity encoding of mechanostimuli by tactile food-sensing neurons requires an ensemble of mechanosensitive ion channels

Ringstad, Niels; Fok, Alice; Brissette, Benjamin; Hallacy, Timothy; Ho, Elver; Ramanathan, Sharad

The nematode C. elegans uses mechanosensitive neurons to detect bacteria, which are food for worms. These neurons release dopamine to suppress foraging and promote dwelling. Through a functional screen of genes highly expressed in dopaminergic food-sensing neurons, we identified a K2P- family potassium channel - TWK-2 - that damps their activity. Strikingly, loss of TWK-2 restores mechanosensation to neurons lacking the NOMPC-like
channel TRP-4, which was thought to be the primary mechanoreceptor for tactile food-sensing. The alternate mechanoreceptor uncovered by TWK-2 mutation comprises a trio of Deg/ENaC channel subunits: ASIC-1, DEL-3, and UNC-8. Analysis of cell-physiological responses to mechanical stimuli indicates that TRP and Deg/ENaC channels work together to set the range of analog encoding of stimulus intensity and to improve signal-to-noise characteristics and temporal fidelity of food-sensing neurons. We conclude that a specialized mechanosensory modality - tactile food-sensing - emerges from the coordination of distinct force-sensing mechanisms housed in one type of sensory neuron.

123. Identification of molecular pathways in neurodegeneration and neuroprotection in nematode excitotoxicity

Mano, Itzhak; Mendelowitz, Zelda; Idrizi, Adem; Chowdhury, Ayesha; Chan, Shirley

Excitotoxicity is a prevalent form of neurodegeneration seen in acute conditions like stroke/brain ischemia and in a rage of progressive neurodegenerative diseases. While we know that excitotoxicity is triggered by excessive accumulation of the neurotransmitter Glutamate (Glu) and hyper-activation of Glu Receptor/Channels (GluRs), our understanding of the process that leads from GluR hyperactivation to necrotic cell death remains highly controversial, and its progression remains untreated in the clinic. Surprisingly, the degenerative process is also partially mitigated by a neuroprotective process that depends on GluR-activated transcriptional programs. The Mano lab focuses on the study of a model of excitotoxicity in the nematode. We use this model to decipher the pathways that lead to necrotic neurodegeneration, and to understand how neurodegeneration is mitigated by transcriptional neuroprotective programs. We have previously identified the cell-death master regulator DAPK as a key mediator of excitotoxic neurodegeneration, and we now see that it works with CEP-1/p53 and induces mitochondrial changes. We also observed that two key transcription factors, CRH-1/CREB and DAF-16/FoxO3, work in concert to induce neuroprotection. We have recently conducted a RNA-Seq study of a subset of neurons that are at risk of degeneration and identified groups of genes whose transcription is modified by both excitotoxicity and CRH- 1/CREB. Based on this analysis, we now identify inhibitory ion channels as mediators of CRH-1/CREB -induced neuroprotection in nematode excitotoxicity. We hope that identifying molecular mediators of neurodegeneration and neuroprotection in nematode excitotoxicity can be informative in the future development of strategies to mitigate neuronal damage in brain ischemia.

124. Immobilization of C. elegans on cultivation plates by thermoelectric cooling for high-throughput submicron-resolution microscopy

Grooms, Noa; Wang, Yao; Jaklitsch, Erik; Schulting, Leilani;Chung, Samuel

Imaging, visual screens, and optical surgery on the nematode C. elegans have produced several groundbreaking discoveries in numerous fields of biology. However, high-resolution in

vivo microscopy approaches require strong immobilization to achieve images free from blur due to animal motion. Immobilization methods require significant manual effort and therefore preclude large-scale studies. In a typical 4-hour microscopy experiment to image 100 animals at high resolution, the work for animal immobilization, mounting, and recovery requires 2.75 hours while only 1.25 hours of this time is used to image. Thus, 2/3rds of the experimental time is not actually spent on imaging.
To improve throughput, we implement a novel cooling method to easily immobilize entire C. elegans populations directly on their cultivation plates. Our technique greatly accelerates imaging as it requires minimal user effort, and it can be easily and inexpensively replicated and utilized by any typical C. elegans laboratory. Based on an upright epifluorescence microscope platform, our cooling methods allows both fluorescence and transillumination brightfield imaging and maintains access to animals during imaging. Compared to standard azide immobilization, cooling immobilization reduces the animal preparation and recovery time by >98%, allowing completion of a typical 4-hour experiment in just over an hour.
We also optimize and characterize the cooling immobilization. Counterintuitively, relatively warmer temperatures, specifically 6 °C, immobilize animals significantly more effectively than colder temperatures utilized in prior studies. This enhanced immobilization enables blur-free submicron-resolution fluorescence imaging, which is challenging to achieve with most current immobilization techniques. We demonstrate 64x magnification 3D imaging and 2-min long timelapse recording of neurons in adults and embryos without any motion blurring. By obviating individual animal manipulation, our approach enables large-scale high-resolution imaging and laser surgery, and it makes in vivo microscopy amenable to automation.

125. In vivo specificity of curvature-sensitive motifs

Bai, Jihong; Zhang, Lin; Wang, Yu; Dong, Yongming; Pant, Aaradhya; Masserman, Laura; Liu, Yan; McLaughlin Jr, Richard

Curvature-sensing mechanisms assist proteins in executing particular actions on various membrane organelles. Here, we investigated the functional specificity of curvature-sensing amphipathic motifs in *Caenorhabditis elegans* through the study of endophilin, an endocytic protein for synaptic vesicle recycling. We generated chimeric endophilin proteins by replacing the endophilin amphipathic motif H0 with other curvature-sensing amphipathic motifs. We found that the role of amphipathic motifs cannot simply be extrapolated from the identity of their parental proteins. For example, the amphipathic motif of the nuclear pore complex protein NUP133 functionally replaced the synaptic role of endophilin H0. Interestingly, non-functional endophilin chimeras had similar defects – producing fewer synaptic vesicles but more endosomes – indicating that the curvature-sensing motifs in these chimeras have a common deficiency at reforming synaptic vesicles. Finally, we converted non-functional endophilin chimeras into functional proteins by changing the cationic property of amphipathic motifs, successfully reprogramming the functional specificity of curvature-sensing motifs*in vivo*.

126. Integration of multisite gating mechanisms in a thermosensory circuit control long lasting behavioral states

Thapliyal, Saurabh; Glauser, Dominique

Animal behavior is a complex integrated output of genes, sensory history, internal state and immediate environmental context. How these factors interact for a single-sensory stimulus to generate long lasting behavioral states remains elusive. Here, we perform high-content analyses of C. elegans behavior to show that steady behavioral states under different conditions can be represented as a unique code of worm posture and locomotion. We found that the response to different long-term thermal history is gated by the nervous system, while change in internal feeding state and thermosensory inputs from the environment generate context-dependent, transient or persistent switch in steady behavioral states. Using behavioral reductionism, we decoded the neuropeptide signals required from tonic thermosensory neurons to make long lasting switch to novel behavioral states. We further demonstrate that tonic sensory neurons modulate baseline calcium levels and neuropeptide expression. Concomitantly, the circuit downstream modulates its responsiveness allowing behavioral state transitions. Our work reveals that persistent transition to distinct behavioral states require integrated lifting of different molecular, cellular and circuit gates across timescales in the nervous system.

127. **Integration of spatially opposing cues by a single interneuron guides decision making**
Gat, Asaf; Pechuk, Vladyslava; Peedikayil Kurien, Sonu; Goldman, Gal; Lubliner, Jazz; Karimi, Shadi; Krieg, Michael; Oren-Suissa, Meital

How do animals efficiently map their environment? C. elegans worms avoid hazardous chemicals they encounter by reversing and changing their direction of movement. The decision whether to move forward or reverse depends on the integration of glutamatergic sensory input from the head and tail by interneurons such as AVA, which is innervated by the head and tail sensory neurons ASH and PHB, respectively. While head sensation elicits calcium activity in AVA and promotes reversals, tail sensation has been suggested to negatively modulate reversals in response to repellents (Hilliard et al., 2002). This study aims to uncover how a single interneuron integrates spatially distinct and opposing cues to tune the animal's behavior. Calcium imaging of AVA interneurons revealed significant inhibition following tail exposure to an aversive cue. We found that this inhibition is mediated specifically by the glutamate-gated chloride channel receptor AVR-14. In avr-14 mutants, inhibition of AVA was completely abolished, and could be rescued by re-expressing AVR-14 specifically in AVA. In double mutants for the AMPA and NMDA-like receptors glr-1 and nmr-1, AVA excitation following head exposure to an aversive cue was abolished while inhibition following tail exposure was intact. Moreover, GFP-labeled AVR-14 localized to the tail synaptic region of the AVA axon, while NMR-1 & GLR-1 localized mostly in anterior regions of AVA process, near ASH synapses. Given the spatial distribution of these receptors, and their subsequent effect on AVA activity, we attempted to resolve their role in regulating avoidance behavior. Double mutants for glr-1 and nmr-1, expressed in AVA, did not reverse in response to a nociceptive cue. Interestingly, triple mutants for glr-1, nmr-1 and avr-14 showed a significant increase in reversal behavior, which could then be canceled by re-expressing AVR-14 specifically in AVA, providing physiological evidence to the role AVR-14 plays in antagonizing reversals. Our data suggests that a single interneuron can integrate multiple spatially- distinct and opposing sensory cues by utilizing different glutamate receptors on distinct regions of its axon.

128. **Investigating how worms integrate sensory and motor information in salt klinotaxis**

Matsumoto, Ayaka; Zhang, Chenqi; Isozaki, Akihiro; Goda, Keisuke; Toyoshima, Yu; Iino, Yuichi

Animals navigate toward their preferred environment by integrating sensory inputs with their own motor state. C. elegans migrates toward NaCl concentrations at which it was cultivated in the presence of food. C. elegans employs several strategies for NaCl chemotaxis, one of which is klinotaxis. Previous studies have found that optogenetic stimulation of ASER, AIY, AIZ or SMB neurons can elicit klinotaxis-like responses. These neurons seem to control the direction of the locomotion through regulating the head bending. However, how the information of NaCl concentration is integrated with motor information to regulate klinotaxis remains unknown. Here we seek to find how sensory and motor information are processed in the neuronal network.
In order to elucidate how the neural circuit integrates NaCl concentration and head bending information, we developed a microfluidic device in which a worm can freely move its head and spatiotemporal NaCl stimuli can be given. Inside the manufactured chip, worms detect NaCl concentration changes during head swings. We found worms bent their heads to ipsilateral side strongly when NaCl concentration changed toward the preferred NaCl concentration. On the other hand, when NaCl concentration changed away from the preferred NaCl concentration, worms bent their heads toward the contralateral side.
We also performed calcium imaging with the manufactured chips and found that the activity of SMBD, the dorsal SMB, not only correlates with head bending but also responds to the NaCl concentration changes in a phase-dependent manner. SMBD responds to the NaCl stimulation only when the activity of SMBD is increasing. This phase-dependent response of SMBD may weaken the ventral head bending. To elucidate the relationship between SMBD and head bending, we performed optogenetic experiments and found that SMBD activation during ventral head bending made ventral bending shallower. These results imply that SMBD regulates head bending.
Together, our results suggest that SMBD integrates sensory and motor information by phase-dependent response and regulates head bending so that worms can navigate toward preferred NaCl concentrations.

129. **Maze learning and 3-dimensional spatial behavior are affected by age and prior experience**

Gourgou, Eleni; Berardi, Lindsay; Fretz, Abrielle; Brooks, Noah; Yang, Ray

C. elegans' ability to exhibit associative learning is well studied, mainly in the context of chemical stimuli. Recently, we demonstrated a new type of associative learning, in which nematodes associate food with a combination of proprioceptive cues and information on the structure of their surroundings (maze), perceived through mechanosensation. By using our custom-made WormMaze platform, made entirely out of NGM, we showed that C. elegans young adults locate food in T-shaped mazes and, following that experience, learn to reach a specific maze arm. Our findings expand what is known about the spatial learning abilities of invertebrate animals. Moreover, we demonstrated that the observed aging-related decline of middle-aged C. elegans maze learning can be reversed by 24-hr food deprivation. Here we show that lifespan-extending genetic manipulations (daf-2 mutants) can prolong healthspan with respect to maze learning. In addition, we show that nematodes cultured in the enriched environment of 3- dimensional agar plates maintain their maze learning ability for longer in their life, in contrast to nematodes grown on standard, flat-surfaced plates. This suggests that

behavioral interventions that pertain to sensory enrichment of an animal's surroundings can play a pivotal role in delaying aging- related deterioration of cognitive-like functions. Furthermore, we postulate that our 3-dimensional culture terrains for C. elegans introduce a novel approach to culturing and experimenting on nematodes in a more naturalistic way than standard methods. To further develop this idea, we introduce our prototype 3D-printer machine, which extrudes NGM agar-based hydrogel for the creation of unique behavioral arenas, suitable for the assessment of C. elegans 3-dimensional behavior. We present findings showing that C. elegans cross 3-dimensional, physical barriers in a way that depends on age and prior experience with similar structures. Our work paves the way for in depth exploration of 3-dimensional animal behavior and characterization of the steering mechanisms.

130. Mechanical compartmentalization of axons governs proprioceptive coordination

Das, Ravi; Lin, Lynn; Català-Castro, Frederic; Malaiwong, Nawaphat; Sanfeliu-Cerdán, Neus; Porta-de-la-Riva, Montserrat; Pidde, Aleksandra; Krieg, Michael

Mechanoreceptors transduce mechanical information into precise biochemical signals by activating mechanosensitive ion channels. In C. elegans during locomotion, proprioceptive neurons sense body postures and provide mechanical feedback to adjust the motor program. Often, these neurons span the animal's whole body, such that sensory neurites are simultaneously stretched and compressed at different sites along the body. How these differential stresses act on cells in moving animals and coordinate ion channel activity over long distances is not understood. Here, we hypothesized that long mechanosensory neurites achieve local computation through mechanical compartmentalization. With the help of conditional, neurons specific CRE/lox screen to disrupt the UNC-70 b-spectrin, we investigated the mechanics, molecules and neurons responsible for proprioception in C. elegans. We identified that alternating tension and compression within the spectrin network of DVA encodes body posture and informs TRP-4/NOMPC and TWK-16/TREK2 homologs of mechanosensitive ion channels during locomotion. With direct visualization of mechanical tension using a genetically encoded FRET-tension sensor, and optical tweezer measurements on isolated neurons in combination with Calcium imaging, we found that DVA becomes activated locally under compressive stresses in vivo and in vitro. In contrast to a widely accepted model of proprioceptive 'stretch' reception, we propose that compression in the neuron leads to compartmentalized activity within long axons delimited by curvature-dependent ion channel activity. Our data sheds light on how mechanical properties of spectrin cytoskeleton stabilize axons against internal stresses and enable force transfer mechanics during locomotion. Since spectrin is highly conserved from worms to humans, our results point towards a conserved primordial mechanism of neuronal stretch signaling of organ volume changes during various

131. Mechanism of state-dependent mechanosensory processing in C. elegans

Kumar, Sandeep; Liu, Mochi; Sharma, Anuj; Leifer, Andrew

A fundamental task of the nervous system is to interpret sensory information in the context of an animal's current actions. The C. elegans nervous system responds to mechanosensory stimuli differently depending on the animal's behavior state. Specifically, a mechanosensory stimulus such as a plate tap delivered to a worm moving forward is more likely to evoke a

reversal response than the same stimulus delivered to a worm when it is turning [1]. The circuit-level mechanism underlying this rapid change in sensorimotor processing, however, remains unknown.
To identify where in the network behavior-state signals are combined and processed together with sensory-related signals, we optogenetically interrogated reversal-associated interneurons downstream of the mechanosensory neurons. We used a high-throughput closed-loop optogenetic delivery system [2] to automatically deliver stimuli triggered to the onset of a behavior such as a turn. We systematically activated RIM, AIB, AIZ, AVE or AVA when the animal was either moving forward or turning. For most of these neurons, optogenetic activation delivered during forward locomotion was significantly more likely to evoke a reversal than activating the same neuron during turning. In contrast, activating AVA evoked reversals with a likelihood that did not depend significantly on whether the animal was moving forward or turning. This suggests that turning information is combined with mechanosensory signals upstream of AVA.
To identify the source of turning related signals, we interrogated the turning-associated neuron RIV which had previously been hypothesized to inhibit reversal neurons [3]. Optogenetic inhibition of neurons RIV, SAAD and SMB abolished the turning dependence of mechanosensory processing.
Specifically, mechanosensory stimulation during turning under simultaneous inhibition of RIV, SAAD and SMB evoked responses that were not significantly different than stimulation during forward movement. This is consistent with an interpretation that RIV may be the source of turning signals that are then combined with mechanosensory signals upstream of AVA.
References

1. Liu, Mochi, et al. Elife (2018).
2. Liu, Mochi, et al. PLoS biology (2022). 3.Wang, Yuan, et al., Elife (2020).

132. Mechanosensation is provided by the E isoform of MEC-2 with a large C-terminal

Keszthelyi, Talia Magdolna; Legradi, Regina; Palya, Dóra; Tory, Kalman

Introduction: The podocin encoding NPHS2 is the most frequently mutated gene in steroid-resistant nephrotic syndrome. We aimed to generate an in vivo model to study the interallelic interactions of podocin. The homologue of NPHS2 in C. elegans is mec-2 encoding several splice isoforms. Among them, MEC-2A has been considered to be the canonical one. The mec-2 null-mutant worms are touch insensitive. Recently (Liang et al. Nucleic Acids Res 2022), two isoforms, MEC-2A and MEC-2E, were described to function in concert and rescue the touch insensitivity only when expressed together. The MEC-2E isoform, also containing a large C-terminal, is significantly longer than the MEC-2A (1239 vs. 481 AA). Furthermore, mec-2 mutants were also found to be insensitive to olfactory stimuli. Our aim was to identify the canonical MEC-2 isoform(s).
Methods: Vectors encoding MEC-2 isoforms under mec-2 promoter and a selection marker (cbr-unc) were generated with NEBuilder DNA assembly kit. To avoid quantitative differences due to extrachromosomal expression, we implemented the MosSCI (Mos1-mediated Single Copy Insertion) technique to achieve chromosomal integration. The mec-2 nonsense mutant (Tu37) strain was kindly provided by the laboratory of Prof. M. Chalfie. Worms were transformed by microparticle bombardment. The gentle-touch sensation was examined by cat's whiskers in a blinded experiment. Chemotaxis assay was performed as described previously by Margie et al. J Vis Exp 2013.

Results: Integrant strains were successfully generated expressing MEC-2A or MEC-2E. While no rescue was achieved by MEC-2A expression, the mechanosensation of the strain expressing the MEC-2E isoform was indistinguishable from that of the wt. In the chemotaxis assays, we could not detect a significant loss of function in the mec-2 null-mutants. **Conclusion:** As the MEC-2E isoform was able to rescue the mechanosensation defect on its own, we conclude that the MEC-2E isoform is the canonical transcript. The large C-terminal of MEC-2E is thus crucial for the mechanosensation function. We detected no relevant olfactory function for MEC-2.

133. Microbial diet-dependent modular glucosides modulate escape responses

Balch, Julia; O'Donnell, Michael

Diet and the gut microbiome have the capacity to impact nervous system function in many ways, however, the molecular features of these interactions are not well understood in any experimental system. A newly discovered class of diet-dependent C. elegans metabolites, modular glucosides (MOGLs), have been hypothesized to play a role in various physiological processes, including in influencing the nervous system. The carboxylesterase enzyme CEST-1.2 is expressed in the intestine and head of worms and it contributes to the assembly of over 150 MOGLs containing tyramine (tyglu), indole (iglu), or anthranilic acid (angl) moieties, suggesting that a function of this enzyme may be in the regulation of these potentially neuroactive groups. One such moiety, the neurotransmitter tyramine, regulates the locomotory escape response of C. elegans following anterior touch stimulus. Worms bearing cest-1.2 mutations exhibit feeding-state dependent defects that are phenotypically similar to worms lacking the ability to produce tyramine or lacking the tyramine-gated chloride channel, LGC-55. Surprisingly, while these behavioral phenotypes indicate a defect in tyraminergic signaling, the cest-1.2 mutant phenotype is also suppressed when bacterially-produced indole is experimentally eliminated. This suggests that an indole-containing compound, likely an iglu, is also required for the typical C. elegans escape response. We theorize that MOGLs produced in the intestine may signal via sensorimotor circuits to regulate escape response as a function of feeding state. Thus, small-molecule signaling via MOGLs presents an exciting model for understanding conserved mechanisms of how microbiota-produced metabolites like indole and tyramine may regulate the nervous systems of animals.

134. Neural modulation of systemic stress response requires the insulin like-peptide INS-3

Veuthey, Tania; Giunti, Sebastian; De Rosa, Maria Jose; Alkema, Mark J; Rayes, Diego

Throughout the animal kingdom, it has been observed that perpetuation of the flight response leads to reduced ability to cope with environmental challenges, a drastic lifespan reduction, and an increase in disease susceptibility. We have recently shown that, in*C. elegans* , the tyraminergic neuron RIM supplies a state-dependent neural switch between acute flight and long-term environmental stress responses (De Rosa MJ, et al 2019). We found that during the flight-stress response RIM neurons release TA, which stimulates the intestinal adrenergic-like receptor TYRA-3. This leads to DAF- 2/Insulin/IGF-1 pathway activation and inhibition of cytoprotective mechanisms not only in the intestine but also in other tissues. We hypothesized that TYRA-3 stimulates the release of Insulin-Like Peptides (ILPs) from the

intestine that can systemically activate the DAF-2 insulin/IGF1 receptors. Since tyramine signaling leads to the activation of the DAF-2/IIS pathway we focused on strong agonists ILPs that are expressed in the intestine (INS-3, -4, -6, - 32, and DAF-28). We found that *ins-3* mutants are resistant to both heat and oxidative stress, much like *tyra-3* mutants. Moreover, *ins-3* mutants are resistant to the impairment of stress resistance upon exposure to exogenous tyramine. In addition,*ins-3;tyra-3* double mutants are as resistant to environmental stress as single mutants, suggesting that both genes act in the same pathway. Since ins-3 is expressed in neurons and the intestine, we performed tissue-specific rescue experiments. We found that expression of ins-3 in the intestine restores stress resistance to wild-type levels. Taken together, our results suggest that intestinal activation of TYRA-3 by the escape neurohormone TA leads to INS-3 release which acts as an endocrine, autocrine, and/or paracrine signal to activate DAF-2 in different tissues. Our data uncover brain-gut communication pathway in which flight stress neurohormones activate the Insulin/IGF-1 pathway and inhibit the induction of cytoprotective mechanisms.

135. **Neurexin isoforms act cooperatively to initiate and maintain foraging activity**

Hart, Michael; Bastien, Brandon

Neurexins are synaptic adhesion molecules with diverse roles in synaptic specification, function, maintenance, and plasticity. The*NRXN1* gene is associated with neuropsychiatric and neurodevelopmental conditions characterized by changes in behavior, including autism spectrum, schizophrenia, Tourette syndrome, and others. *NRXN1, NRXN2,* and *NRXN3* encode alpha and beta isoforms, while *NRXN1* also uniquely encodes a gamma isoform with no known role in behavior. We find that both gamma and alpha *nrx-1/NRXN1* are required for the *C. elegans* behavioral response to food deprivation, a sustained increase in activity for foraging. We find that gamma NRX-1 contributes to initiation, while alpha NRX-1 regulates maintenance, of the increased activity upon food deprivation. We confirm a role for octopamine in increasing activity levels in response to lack of food, and uncover a novel requirement for *nrx-1* in the response to octopamine signaling. Expression of gamma NRX-1 in the octopamine producing RIC interneurons fully restores the behavioral response to food deprivation. We find the maintenance role of alpha NRX-1, but not the initiation role of gamma NRX-1 (which lacks synaptic extracellular domains), is conditional on the presence of a neurexin transsynaptic binding partner neuroligin/*nlg-1* . Collectively, our results uncover a novel role for gamma NRX-1 in octopamine signaling and the generation of behavior, as well as cooperative functioning of the conserved alpha and gamma neurexins to initiate and maintain foraging behavior.

136. **Neuroligin-mediated neurodevelopmental defects are induced by mitochondrial dysfunction and prevented by lutein in C. elegans**

Ventura, Natascia; Maglioni, Silvia; Melcher, Marlen; Brinkmann, Vanessa; Luo, Zhongrui; Laromaine, Anna; Schiavi, Alfonso; Raimundo, Nuno; Meyer, Joel; Distelmaier, Felix

Complex-I-deficiency represents the most frequent pathogenetic cause of human mitochondriopathies. Therapeutic options for these neurodevelopmental life-threating disorders do not exist, partly due to the scarcity of appropriate model systems to study them. In our recent work (Maglioni et al. Nat Comm, 2022), we generated new C. elegans models for

mitochondriopathies and showed that depletion of complex I subunits recapitulates biochemical, cellular and neurodevelopmental aspects of the human diseases. We exploited two of these models, nuo-5/NDUFS1- and lpd- 5/NDUFS4-depleted animals, for a suppressor screening that identified lutein for its ability to rescue animals' neurodevelopmental deficits. We uncovered overexpression of synaptic neuroligin as an evolutionarily conserved consequence of mitochondrial dysfunction, which we find to mediate an early cholinergic defect in C. elegans. We showed lutein exerts its beneficial effects by restoring neuroligin (nlg-1) expression independently from its antioxidant activity, thus pointing to a possible novel pathogenetic target for the human disease. As we also found increased expression of nlg-1 to be causally involved in the pro-longevity effect promoted by lutein (Maglioni et al. Cells, 2022), we speculate this synaptic protein may function as a cellular rheostat modulating neuronal health in a context-specific manner.

137. Neuronal activity state governs the decline of cognitive plasticity with age

Busch, Emanuel

Neural plasticity, which is the basis or learning and memory formation, declines continuously and progressively over the course of life. However, the molecular mechanisms underlying the decline of learning and neural plasticity with age are not well understood. Sensory input and neural excitation have been shown to regulate organismal ageing processes and control lifespan, but how they drive the ageing of cognitive function and plasticity remains obscure.
We use the robust behavioural responses mediated by the URX, AQR and PQR neurons to ambient O2 to elucidate how neural plasticity is altered in ageing animals. We discovered that long-term high neural activity of the O2-sensing neurons accelerates the decline of experience-dependent plasticity with age at both the neuronal and behavioural level. In contrast, sustaining lower activity of the O2-sensing neurons retains their plasticity with age.
Gene expression profiling of the O2-sensing neurons in ageing animals shows that neuronal activity alters age-related changes in transcription. Our data suggest that high-activity neurons redirect their resources from maintaining plasticity to sustaining continuous firing. In particular, the differential expression of neuronal genes that modulate Ca2+ homeostasis points to their role in mediating the decline of neuronal plasticity with age.
Our findings demonstrate that the activity of neurons alters neuronal homeostasis to govern the decline of neural plasticity with age.

138. Neuronal polarity requires an endocytic clearance mechanism in the axon initial segment

Eichel, Kelsie; Shen, Kang; Taylor, Caitlin

Neurons are highly polarized cells that face a fundamental challenge of compartmentalizing a vast and diverse repertoire of proteins in order to function properly. The axon initial segment (AIS) is a specialized domain separating a neuron’s morphologically, biochemically, and functionally distinct axon and dendrite compartments. How the AIS maintains polarity between these compartments is not fully understood. Using the extremely morphologically polarized C. elegans PVD sensory neuron, we identified hallmark features of an AIS in neuronal polarity. Most notably, we found a specific subcellular enrichment of UNC-44, an ortholog of the AIS master organizer ankyrinG, between the axonal and dendritic domains. We then

elucidated a novel function of the AIS in neuronal polarity that is conserved from C. elegans to humans. We found that dendritically and axonally polarized transmembrane proteins are recognized by endocytic machinery in the AIS, robustly endocytosed, and targeted to late endosomes for degradation. Forcing receptor interaction with ankyrinG antagonizes receptor endocytosis in the AIS, causes receptor accumulation in the AIS, and leads to polarity deficits with subsequent morphological and behavioral defects. Our results reveal a conserved endocytic clearance mechanism in the AIS that is essential for neuronal polarity and identify AIS endocytosis as a strategy to reinforce axonal and dendritic compartment boundaries.

139. Nuclear hormone receptor NHR-49 in the oxygen-sensing neurons mediate pathogen avoidance in C. elegans

Yoon, Kyoung-hye; Kwon, Saebom

Both fight and flight are important to survive in a harmful environment. In C. elegans, the nuclear hormone receptor NHR-49 is a functional homolog of mammalian PPAR(peroxisome proliferator-activated receptor), and serve as an important regulator of fat metabolism. In addition to altered lipid composition, nhr-49 mutants display a pleiotropy of defects, including shorter lifespan, impaired starvation response, and increased susceptibility to oxidative stress and pathogenic bacteria, hinting at the diverse roles that lipids play in the body. While trying to understand how NHR-49 controls immunity, we found that a significant part of the mutant's susceptibility to Pseudomonas aeruginosa (PA14) was due to defective avoidance response to the pathogenic lawn. Restoring NHR-49 in the neurons significantly improved avoidance, whereas intestinal rescue did not. Among the neurons, we found that restoring NHR-49 expression in cholinergic and glutamatergic neurons was sufficient for the increased avoidance. Genetic studies showed that NHR-49 acted downstream of the TGFß/DAF-7-mediated chemosensory detection of PA14, as well as the induction of NPR-1 ligand neuropeptides previously shown to elicit pathogen avoidance. Since NPR-1 is known to signal through a neuronal circuit that includes the oxygen-sensing neurons, we restored NHR-49 selectively in the oxygen-sensing AQR, PQR and URX neurons. We found that this was sufficient for the increased avoidance, but avoidance was not due to preference for any oxygen concentrations. We are currently trying to understand how NHR-49's role as a lipid regulator influences neuronal function and behavior.

140. Orthogonal Signaling Shapes Sexually Dimorphic Branching and Functions of PVP Interneurons in C. elegans

Chen, Rui-Tsung

Animals display sexually dimorphic behaviors partly through assembling sex-specific circuits with different neuronal structures. Despite previous studies centered on the effects of the intrinsic program on neuronal structures, it is unclear whether neurons orchestrate external cues to shape sexually dimorphic morphogenesis. In this study, we show that sex-shared PVP cholinergic interneurons display sexually dimorphic collateral branching. We find that PVP collateral branches emerge near the vulva at late larva stages in hermaphrodites, while PVP branching does not appear in adult males.
Interestingly, branch morphology is highly dynamic regulated by nutritional status through DAF-16/FOXO transcription factor, suggesting a role of PVP branches in environmental

adaptation. Genetic analysis reveals that the vulva is essential and sufficient to promote branch formation, but the degree of branch formation depends on sexual identity. Lastly, functional characterization of PVP branches demonstrates a sex-specific role in sex-specific egg- laying circuit. Together, we propose that intrinsic sexual identity modulates the sensitivity of sex-shared neurons to external growth factors that ultimately establish sexually dimorphic structures for sex-specific circuits. Thus, our study supports a model that orthogonal signaling reconciled by internal and external signaling controls sexually dimorphic morphogenesis in C. elegans.

141. Pathways regulating C. elegans exercise-induced fatigue during prolonged swimming activity

Schuch, Kelsey; Hart, Anne

Fatigue is poorly understood beyond muscle energetics. For *C. elegans* , swimming is a more energetically costly behavior than crawling and can be classified as a form of exercise (PMID 28395669). After prolonged swimming, *C. elegans* cycle between active and inactive bouts (PMID 19011210); inactive bouts may be sleep or quiescent rest. Sleep is the most well characterized period where*C. elegans* show locomotion quiescence; however, we have shown that the quiescent bouts observed after prolonged swimming exercise do not fully fit the behavioral criteria required to be classified as sleep (PMID **32811254**). It is likely that this locomotion quiescence is a fatigue-induced state of quiescent rest. Using a computer vision program and other approaches we also tested the role of several genes previously implicated in other behavioral quiescence to determine whether they also affect quiescent bouts induced by prolonged swimming. We have found that while there is some overlap between genes involved in regulating behavioral quiescence during *C. elegans* sleep and exercise-induced quiescence, distinct pathways underlie these behaviors. Ultimately, we hope to better understand the pathways involved in regulating fatigue and quiescent bout cycling.

142. Peroxisomal Lipid Metabolism Regulates Stress-Induced Bacterial Avoidance in C. elegans

Tsai, Shang-Heng; Pan, Chun Liang; Wu, Yu Chun

Physiological perturbation can trigger aversive learning that alters animal behavior for avoiding environmental hazards and optimizing survival. In C. elegans, mitochondrial disruption induces learned avoidance common food bacteria through the formation of aversive memory. We found that among genes upregulated upon mitochondrial disruption were several in the peroxisomal lipid metabolism, including pmp-4, which encodes the transporter of very long chain fatty acid (VLCFA), and daf-22 and dhs-28, enzyme genes for fatty acid β-oxidation. Genes in the peroxisomal β-oxidation pathway were required for bacterial avoidance under mitochondrial disruption, and they functioned in the intestine and hypodermis but not neurons. Our genetic experiments suggest that peripheral signals from increased peroxisomal lipid metabolism may target the serotonergic circuit to promoter bacterial avoidance under stress. These data provide novel insights into gut-brain signaling mechanisms that enable stress-induced aversive learning.
supported by the Ministry of Science and Technology, MOST 109-2320-B-002-019-MY3, MOST 110-2320-B-002-057-MY3

143. Pharmacological targeting of C. elegans Two-pore Domain Potassium Channels

Chen, Li; Beets, Isabel; Schafer, William; R. Schafer, William

Parasitic diseases caused by helminths are a serious health issue, particularly in tropical areas. Existing commercial anthelmintics, such as ivermectin and levamisole, act as agonists of ligand-gated ion channels. To combat emerging nematicide resistance due to overuse, it is desirable to identify new channel targets and ultimately to identify novel anthelmintics. Two-pore domain potassium (K2P) channels, a less-extensively studied ion channel family, could be good candidates for new nematicide targets. K2P channels have evolved and diversified independently in different animal phyla, and most of the 47 K2P channels found in C. elegans have no orthologue among human K2P channels. Thus, compounds targeting nematode-specific K2P channels might have anthelminthic potential.
One promising K2P channel is TWK-18, which is expressed in body wall muscles and a small number of sensory and interneurons. Previous work has shown that gain-of-function mutants show severe locomotory defect with elevated temperature, and strong dominant alleles are lethal as homozygotes. Thus, agonists of TWK-18 might be expected to lead to lethal paralysis of nematodes. As a first step to find such compounds, we have heterologously expressed twk-18::gfp in HEK239T cells, and observed robust plasma membrane localization. Patch clamp recordings of these cells reveal temperature-sensitive currents that are blocked by a general K2P antagonist, indicating functional expression of the channel. We next plan to conduct a high throughput agonist screening by using a voltage-sensitive dye to detect activation. Meanwhile, we are also interested in deciphering the role(s) of TWK-18 in neural circuits and behaviors in C. elegans. Single-cell RNA sequencing data indicates TWK-18 is expressed heavily in PVC neuron – a command interneuron for forward locomotion, which receives synaptic inputs from a temperature-responsive neuron. We have generated a twk-18 null allele using CRISPR and are currently investigating the roles of twk-18 in locomotion and temperature-related neural circuits.

144. Phosphorylation of CMK-1 on T179 promotes both nuclear entry and export in a tonic nociceptor neuron upon prolonged activation

IPPOLITO, DOMENICA; Glauser, Dominique

Nociceptor neurons detect noxious stimuli, as potential source of harms, and transmit the information in the nervous system to trigger essential avoidance behaviour. In a situation of concurrent exposure to multiple stresses, the capacity to modulate the sensitivity threshold for nociception is necessary to maintain the ability to respond to other, maybe more threatening, stimuli. We look at C. elegans adaptation to noxious heat as a paradigm to study nociception plasticity. We previously showed that a prolonged exposure to a mild noxious temperature (28°C for 1 hour) leads to a desensitization of the avoidance response, which depends on the translocation of Ca2+/Calmodulin-dependent protein kinase-1 (CMK-1) into the nucleus of FLP thermo-nociceptor neurons. The binding of the calcium/calmodulin complex to CMK-1 is one of the events promoting its nuclear import via a quantitative up-regulation of importin binding. The phosphorylation of Thr179, which is common to other CaM kinases, seems to be involved as well, but its effect is poorly understood. Here, we dissect the contribution of T179 to CMK-1 subcellular localization by using fluorescently tagged CMK- 1

proteins with phospho-mimic (T179D) or phosphorylation-dead (T179A) mutations, alone or in combination with a series of additional mutations. We found that T179 phosphorylation is required and sufficient to promote CMK-1 nuclear entry, working in synergy with CaM-binding and converging on the exposure of the same Nuclear Localization sequence (NLS). Surprisingly, we found that T179 phosphorylation also promotes an antagonistic nuclear export drive. Collectively, our data suggest a model in which T179 phosphorylation releases two gates, on import and export respectively, in order to set CMK-1 in an active nucleo-cytoplasmic shuttling regime, thereby enabling other factors, such as intracellular calcium concentration, to quantitatively tune the import/export kinetics and alter CMK-1 localization at equilibrium.
Overall, our work elucidates a multi-level regulatory mechanism, which, given the widespread expression and high conservation of CaM kinases, could be relevant for other C. elegans adaptable behaviours and in tonic neurons in other species.

145. PIM-related kinases of C. elegans have pleiotropic roles in neuronal and developmental processes.

Koskinen, Päivi J; Kalichamy, Karunambigai S; Hudson, Martin L; Tuomaala, Joel

The mammalian PIM family of serine/threonine kinases regulate several cellular functions, such as cell survival and motility, and are implicated in multiple types of cancer. As PIM-related kinases (PRKs) are well conserved across phyla, it is possible to study their functions in model organisms, such as C. elegans nematodes which express two PRK proteins, PRK-1 and PRK-2.
In our previous studies, we have shown that chemical inhibitors of mammalian PIM kinases block activities of also C. elegans PRKs (Kalichamy et al., eNeuro 2019). Moreover, these inhibitors interfere with chemotactic movements of the nematodes in response to olfactory stimuli sensed by the AWB and AWCON neurons, suggesting that PRKs play an important role in the olfactory circuits.To confirm these findings and to elucidate other physiological functions for PRKs, we examined the behaviour of two loss-of-function mutant strains, prk-1(pk86) and prk-2(ok3069). There wenoticed that prk-1 mutants displayed not only olfactory, but also developmental defects along with a remarkably reduced brood size, while prk-2 mutants were less severely affected. However, both prk-1 and prk-2 mutations resulted in abnormal forward locomotion. More detailed morphological and life-span analyses revealed that prk-1 is essential for proper body length, body bends, male tail development as well as longevity. In addition, it has a role in neuronal development, as prk-1 mutants showed defects in AIY interneuron axon outgrowth. The differential impacts of the mutations may be explained by differences in prk expression patterns.
Using reporter genes, we observed that both prks are expressed in the intestine, while prk-1 is strongly expressed in the nervous system and prk-2 in epithelial seam cells. Work is currently inprogress to rescue the loss-of-function phenotypes using transgenic approaches.

146. Post-developmental roles of the netrin receptor UNC-40/DCC mediated by a novel phosphodegron motif

Salzberg, Yehuda; Sela, Sapir; Oren-Suissa, Meital

UNC-6/Netrin and its receptor UNC-40/DCC are mostly studied for their developmental roles in neuronal navigation. Much less is known about their roles in the mature nervous system, despite the known genetic association of DCC variants with many neurological conditions in

adult humans. We report two post-developmental processes in C. elegans that are regulated by UNC-40 (homolog of DCC) and require a conserved phosphodegron motif located in its cytoplasmic tail.
First, we found that UNC-40 acts to maintain specific synapses in adult male worms. In hermaphrodites, however, UNC-40 is degraded by the conserved E3 ubiquitin ligase SEL-10/FBW7, thus leading to the sex-specific removal of these synapses. We further defined a conserved phosphodegron sequence within UNC-40 that mediates the binding of SEL-10 and show that mutating this sequence in worms prevents UNC-40 degradation and stabilizes the synapses even in adult hermaphrodites.
Second, we found that UNC-40 promotes the loss of dopaminergic (DA) neurons in aC. elegans model for neurodegeneration (induced either by 6-OHDA or expression of human alpha-synuclein). In UNC-40-null animals, DA neuron degeneration was attenuated. Remarkably, in animals with a mutated UNC-40 phosphodegron, DA cell degradation was significantly enhanced and spontaneously appeared even without 6-OHDA treatment, in a mechanism involving the parthanatos alternative cell death pathway.
Together, our results reveal new roles for UNC-40/DCC in the mature nervous system and identify a conserved motif that mediates these functions.

147. **Reconstructing the evolutionary history of C. elegans neuropeptidergic systems**

Geens, Ellen; Van Damme, Sara; Beets, Isabel; Schoofs, Liliane; Temmerman, Liesbet; Vandewyer, Elke; Zels, Sven; Mirabeau, Olivier; Golinelli, Luca

Environmental changes necessitate continuous adaptation of an animal's physiology and behavior. While synaptic communication plays a pivotal role in this process, neuropeptides represent the largest group of neuromodulators that regulate adaptive behaviors including feeding, reproduction, and learning. Many of these neuropeptidergic systems are evolutionary ancient and can be found across the Animal Kingdom. In C. elegans, over 150 neuropeptide receptor and neuropeptide genes have been identified. However, the evolutionary history and neuropeptide-receptor interactions for most of these signaling systems remain elusive.
To gain insight into the conservation and evolution of C. elegans neuropeptide-receptor couples, we performed a large-scale phylogenetic analysis and deorphanization of neuropeptide G protein-coupled receptors (GPCRs). Out of 31 ancient neuropeptide receptor families, which are conserved across bilaterian animals, we found 17 to be present in C. elegans. These include neuropeptide systems related to well-known vertebrate systems, such as tachykinin, neuropeptide Y and neuromedin U. For most of the conserved GPCRs, we discovered that the receptor is activated by a ligand from the same neuropeptide family, supporting evolutionary conservation of these signaling systems. In addition, our results highlight several neuropeptide- receptor families that have largely expanded in nematodes. Among these, we discovered a diversified group of nematode GPCRs related to bilaterian somatostatin (SST) and opioid receptors. Deorphanization of these receptors revealed that they are activated by both known and newly predicted orthologs of the SST neuropeptide family. These findings support the co-evolution and expansion of SST-like GPCRs in C. elegans. Taking advantage of the well characterized nervous system of C. elegans and the many tools available for biochemical and functional studies, we are building upon these findings to uncover the function and signaling network by which they act as neuromodulators. This will

further aid in unraveling evolutionarily conserved neuropeptide pathways important to brain function.

148. Sexually dimorphic architecture and function of a mechanosensory circuit

Setty, Hagar; Salzberg, Yehuda; Karimi, Shadi; Krieg, Michael; Oren-Suissa, Meital

How sensory perception is processed by the two sexes of an organism is still only partially understood. Despite some evidence for sexual dimorphism in auditory and olfactory perception, whether touch is sensed in a dimorphic manner has not been addressed. Here we deconstructed the neuronal circuit for tail mechanosensation in C. elegans and found it is wired differently in the two sexes and employs a different combination of sex-shared sensory neurons and interneurons in each sex. Reverse genetic screens uncovered cell- and sex-specific functions of the alpha-tubulin mec-12 and the ion channel tmc-1 in sensory neurons, and of the glutamate receptors nmr-1 and glr-1 in interneurons, revealing the underlying molecular mechanisms that mediate tail mechanosensation. Moreover, we show that only in males, the sex-shared interneuron AVG is strongly activated by tail mechanical stimulation, and accordingly is crucial for their behavioral response. Importantly, sex reversal experiments demonstrate that the sexual identity of AVG determines both the behavioral output of the mechanosensory response and the molecular pathways controlling it. Our results demonstrate that the propagation of harsh-touch tail mechanosensory information is sexually dimorphic, and provide a unique example of how neuronal circuits evolved sex- specific features while maintaining the same sensory modality and its behavioral output.

149. Specific (co-)transmission of two neuropeptide species from the AVK interneuron

Aoki, Ichiro; Gottschalk, Alexander

Communication among cells via neuropeptides is crucial for proper function of the nervous system, as is evident from human diseases caused by disruption of neuropeptide signaling. For example, lack of Neuropeptide Y is involved in a variety of disorders such as hypertension, epilepsy, and obesity (Reichmann 2016; Yi 2018). Across diverse species, many neurons express multiple neuropeptides and small molecule transmitters, raising the question whether these neurons simply co-release cocktails of transmitters, or whether some of those neurons may release transmitters separately and specifically.
We previously found that the AVK interneurons of C. elegans may specifically (co-)transmit at least two different neuropeptides, FLP-1 and NLP-49. Both are expressed at high levels and predominantly in AVK and differentially affect behaviors such as locomotion and egg-laying (Oranth 2018; Chew 2018). However, it remains largely unclear whether, in what context and by what mechanisms their release is differentially regulated. We thus examined whether these peptides are differentially regulated. We found from locomotion analyses using the multi worm tracker (Swierczek 2013) that these peptides derived from AVK oppositely affect locomotion and that FLP-1 affects the animal's response to sensory stimuli in a modality-specific manner. We also observed peptide precursors fused with fluorescent proteins and obtained evidence indicative of differential trafficking and regulation of those two neuropeptides in AVK.
We recently found that optogenetic activation of AVK accelerates locomotion, which is remarkably enhanced in the absence of FLP-1. The acceleration was suppressed by tetanus

toxin light chain (TeTx) but not by the loss of *nlp-49,* indicating that some other transmitter(s) released from AVK mediates acceleration. We are currently trying to identify the transmitter responsible for the acceleration, by AVK-specific RNAi of candidate transmission pathways, and studying the neural circuit downstream of AVK that is responsible for the acceleration by whole brain imaging combined with optogenetic AVK-activation.

Reichmann F. and Holzer P. (2016) Neuropeptides Yi M. et al. (2018) Cell Physio. Biochem Chew, Y. L. et al. (2018) Philos Trans R Soc Lond B Biol Sci 373(1758). Oranth, A. et al. (2018) Neuron 100(6): 1414-1428 e1410.
Swierczek, N. A. et al. (2011) Nat Methods 8(7): 592-598.

150. Temperature-regulated gene expression changes drive plasticity in the AFD thermosensory neurons

Harris, Nathan; Bates, Samuel; Zhuang, Zihao; Bernstein, Matthew; Calarco, John; Sengupta, Piali

Animals use information gained from prior experiences to inform future decisions. Integration of experiences requires long-lasting neuronal plasticity driven in part by experience-dependent alterations in gene expression. A remarkable feature of plasticity-associated neuronal gene expression changes is that they can precisely reflect the temporal dynamics or magnitude of stimuli. Activity-dependent gene expression changes have largely been addressed in central circuits, but whether similar forms of plasticity also operate in sensory neurons is unknown. Moreover, few studies have causally linked activity-dependent gene expression changes in defined neurons to behavioral plasticity*in vivo* . Our goal is to develop a model featuring a precise behaviorally relevant stimulus, single neuron measurements of gene expression, and quantifiable neuronal plasticity, and to characterize underlying molecular and neuronal mechanisms.

Well-characterized plasticity of responses in the AFD thermosensory neuron pair allows *C. elegans* to alter their preferred temperature based on prior temperature experience. We previously uncovered components of a transcriptional pathway regulating plasticity in AFD, suggesting AFD could be a useful model for understanding experience-dependent gene expression mechanisms. We measured the temperature experience-dependent transcriptome of AFD using translating ribosome affinity purification (TRAP) with GFP-tagged ribosome subunits expressed in AFD, followed by RNA- Seq. Using primarily endogenous reporters, we characterized the expression of selected genes, finding differences in their temporal dynamics.

Additionally, we identified genes whose expression levels appear to report either magnitude of temperature change or absolute temperature. Levels of the *C12D8.15* -encoded novel transmembrane protein are regulated by high magnitude temperature changes. C12D8.15 resides at the sensory endings of AFD, and is required for experience-dependent modulation of AFD thermosensory responses and thermotaxis behavior. In contrast, expression levels of the *dac-1* Dachsund transcription factor appears to report absolute temperature, and modulates the dauer developmental decision via AFD.

We have also established that only a subset of temperature-regulated gene expression changes requires the activity-dependent transcriptional regulators CaM kinase/CMK-1 and/or CREB/CRH-1 (see abstract by Sam Bates), implying complex gene regulatory programs. We will use this system to understand how temperature, a continuous variable, induces analog gene

expression changes, enabling plasticity in AFD that precisely matches the animal's recent and past temperature experience.

151. The ARID-type transcription factor CFI-1 controls functional features of hub interneurons in the C. elegans posterior touch circuit

Marques, Filipe; Kratsios, Paschalis

Mechanosensation is the process associated with the detection and transmission of mechanical forces by mechanoreceptors into the nervous system. This is a vital sensory modality that allows most animals to discriminate different mechanical cues and to generate appropriate behavioral responses, like touch-reflex or avoid harmful mechanical stimuli. Mechanosensation relies on the establishment and maintenance of a functional touch circuit, typically composed of sensory neurons that detect mechanical stimuli, interneurons involved in signal processing, and motor neurons that drive muscular activity and behavioral output. Hence, the function of the touch circuit critically relies on mechanisms that ensure the establishment and maintenance of functional features (e.g., neurotransmitter phenotype, electrical properties) of all these interconnected neuron types. To reveal such mechanisms, we focus on the transcriptional control of "terminal identity genes", which encode proteins necessary for neuronal function (e.g., enzymes and transporters involved in neurotransmitter biosynthesis, neurotransmitter receptors, ion channels, neuropeptides).
Previous studies and our work revealed that the conserved ARID-type transcription factor CFI-1 is expressed in sensory (PDE), inter-(AVD, PVC, LUA) and motor neurons (DA, VA, DB, VB, DD, VD) of the touch circuit, leading us to hypothesize that a single transcription factor controls the terminal identity of all interconnected neurons within the circuit, thereby ensuring mechanosensation. Through behavioral experiments in*cfi-1* null mutant animals and conditional knockdown strains, we found that CFI-1 is required continuously, from development to adulthood, for normal posterior touch response behavior. Moreover, depletion of CFI-1 specifically in motor neurons did not result in touch response defects, suggesting the gene is required in either sensory or interneurons of the circuit. Supporting the latter, we identified scores of terminal identity genes as putative targets of CFI-1 in the PVC and LUA interneurons. ChIP-Seq data for CFI-1 support a direct mode of regulation of terminal identity genes in these interneurons. Finally, we are employing a candidate approach focused on other transcription factors (UNC-3, EGL-5, and CEH-14) expressed in these neurons to assess whether and how they collaborate with CFI-1. This study will contribute to our understanding of gene regulatory mechanisms that establish and maintain the functionality of a touch circuit.

152. The C. elegans precipice response is influenced by force vectors and experience

Young, Jared; Mitchell, Robin; Zhang, Shuyu; Pattillos, Diana; McCoy, Savannah; Pulice, Will; Hodnett, Katja

The precipice response in C. elegans is a behavioral phenomenon wherein a worm will strongly reverse upon the first encounter of its nose with the edge of an agar chunk. Although mentioned in a Wormbook section on Mechanosensation Behavior written by Martin Chalfie (Hart, WormBook 2006), until recently this behavior remained uncharacterized. Our previous work defined the behavior as a reversal within two seconds of the nose tip moving past the

edge of an agar chunk that must complete at least one full sine wave and showed that mechanosensation—and in particular, anterior harsh touch sensation—is required for normal rate of precipice response. We designed precipice assays to compare N2 wild-type worms to three mutant strains with varying levels of touch deficiency: mec-3(e1338), mec-10(e1515), and trp-4(sy695) and found that only mec-3 worms with total loss of gentle and harsh touch sensation had a significantly decreased rate of precipice response (Mitchell et al., Micropublication Biology 2021).
We will report on additional experiments we have done to further elucidate the mechanisms of the precipice response in C. elegans. We have found that the precipice response is elicited more frequently when the worms are on top of an agar chunk (and their head goes over the chunk edge), than when they are on the side of a chunk. This indicates that the response is influenced by the particular force vectors experienced by the head, and not solely by the experience of the head losing contact with the agar surface. We have also found that the precipice response is elicited less frequently when the worms are not raised on completely flat agar plates, but rather in an environment in which there are enriched opportunities for the worm to experience the edge of an agar chunk, showing habituation of the response. We also hope to report on experiments in progress, in which we are testing a variety of mutants and genetic ablation lines with the aim of identifying the specific neurons and sensory modalities involved in the response.

153. **The C. elegans touch receptor neurons can signal the unavailability of touch information in touch-deficient worms**

Rabinowitch, Ithai; Staum, Michal

Sensory loss often elicits compensatory behavioral adjustments. We have previously shown that in C. elegans, loss of mechanosensation in the touch receptor neurons (TRNs) results in enhanced olfactory sensitivity, through a FLP-20 neuropeptide-dependent increase in AWC→AIY transmission. In addition to such enhanced sensitivity, we have now found that loss of touch elicits also various cautious-like behaviors. For example, touch-deficient worms show reduced dispersal when removed from food. This behavioral modification too, is associated with decreased TRN secretion of FLP-20. We expected that complete loss of the TRNs, and not just dysfunctional touch sensation, would further reduce the FLP-20 signal and produce even less dispersing. Surprisingly, we found instead that removal of the TRNs abolished behavioral adjustment, suggesting a role for the TRNs in signaling touch insensitivity. We further found that whole-worm FLP-20 transcription decreased in touch-deficient worms with intact TRNs, but was normal in worms lacking the TRNs. Strikingly, these differences in FLP-20 transcription could be traced to the ASE salt-sensing neurons, which similarly to the TRNs, express the FLP-20 neuropeptide. Consistently, we observed enhanced salt sensation in touch deficient worms compared to normal worms and worms lacking the TRNs. These results suggest a form of neuropeptide-based cross-sensory cooperation between FLP-20 secreting neurons, requiring the TRNs for signaling the loss of touch sensation and enabling worms to adjust their behavior to the unavailability of touch information.

154. **The mind of a dauer: Deviations in mitochondrial morphology in neuromuscular system revealed by deep learning-based EM reconstruction**

Bae, J. Alexander; Ko, Gwanho; Ahn, Soungyub; Yim, Hyunsoo; Choe, Daniel T.; Nguyen, Ken C.; Kang, Hae Mook; Bahn, Sang-kyu; Hall, David H.; Kim, Jinseop S.; Lee, Junho

Mitochondria are highly dynamic organelles that constantly alter their shape and position according to the needs such as synaptic transmission. Behavioral responses in different developmental stages vary. Therefore, mitochondrial morphology is expected to differ between different stages, especially in the alternative developmental stage, the dauer. 3D electron microscopy (EM) provides diverse information on cells, circuits, and organelles in detail. We developed semi-automated methods for reconstructing synapses and mitochondria in C. elegans EM images using deep learning.
Consequently, we assembled connectivity graph and mitochondria reconstructions in C. elegans dauer. Moreover, we collected mitochondria reconstructions in normal reproductive stages with published EM datasets (Witvliet et al., 2021), enabling comparative study on morphology and arrangement of mitochondria between different stages. As previous reports, we have validated spatial distribution of mitochondria in neurons is related to the spatial distribution of presynaptic sites using our reconstructions, which indicates mitochondria could help infer the connectivity of neurons. We discovered mitochondria in motor neurons are significantly overrepresented in the dauer stage compared to the non-dauer stages. Since mitochondria are related to synapses, this means motor neurons have an increased role in the dauer nervous system. Lastly, we inspected mitochondria in the body wall muscles as muscles are controlled by the nervous system, causing behavioral responses. Mitochondria in body wall muscles exhibit highly stranded network structure, similar to the mitochondria in adults, while mitochondria in L2 or L3 do not show this special structure. This result has been verified with light microscopy in different developmental stages, including L4 after dauer. We believe these deviations in mitochondrial morphology observed from C. elegans dauer imply changes in the neuromuscular system, which could explain the dauer-specific behaviors.
Witvliet, D., Mulcahy, B., Mitchell, J.K., Meirovitch, Y., Berger, D.R., Wu, Y., Liu, Y., Koh, W.X., Parvathala, R., Holmyard, D., et al. (2021). Connectomes across development reveal principles of brain maturation. Nature 596, 257–261.

155. **The neural basis of heat seeking in a human-infective parasitic nematode**
Bryant, Astra; Hallem, Elissa; Ruiz, Felicitas;Lee, Joon Ha

Soil-transmitted parasitic nematodes infect over a billion people and cause devastating morbidity, primarily in the world's most socioeconomically disadvantaged communities. Previous studies from our lab and others have shown that parasitic worms actively locate human hosts using body heat. However, virtually nothing was known about the neural adaptations that enable parasitic worms to target humans.
We investigated the neural basis of temperature-driven host seeking in parasitic nematodes using Strongyloides stercoralis, a potentially fatal skin- penetrating human parasite that infects at least 610 million people globally. We investigated the molecular and cellular mechanisms underlying temperature-driven host seeking in parasitic nematodes using S. stercoralis. Using CRISPR-Cas9 mutagenesis, we found that heat seeking by S. stercoralis infective larvae (iL3s) is dependent on a cGMP signaling pathway that is conserved across free-living and parasitic nematodes. We identified the primary thermosensory neurons in S. stercoralis and characterized their responses to thermal stimuli by applying single-cell genetic targeting, cell- type specific neural silencing, and genetically-encoded fluorescent biosensors

for the first time in any endoparasitic animal. These neurons display unique thermal response properties that support the ability of parasitic worms to engage in long-distance host targeting using body heat. We investigated the molecular substrates that contribute these unique response properties: we identified the thermoreceptor proteins confer parasite- specific sensitivity to body heat, and revealed evidence that additional molecular elements of the cGMP signaling cascade are regulated by temperature in a parasite-specific manner.
Together, these results are the first direct evidence that the sensory neurons of parasitic worms exhibit unique molecular adaptations that allows them to target humans, a finding with important implications for the evolution of parasitic behaviors in nematodes and efforts to develop new therapeutic strategies for nematode control.

156. The role of novel identified regulator, SFXN-1.2 in mitochondrial dynamics in neurons and establishing linked neurological disease models

Barmaver, Syed Nooruzuha; Wagner, Oliver

Various neurological diseases are linked to changes in mitochondrial dynamics in neurons. Thus, it is critical to understand how the dynamics of mitochondria are regulated on the molecular level. From a candidate screen (95 genes), we have identified a novel gene called sfxn-1.2 (ortholog of human Sideroflexin 1/3), a mitochondrial protein enriched in neurons associated with Alzheimer's disease and Parkinson's disease. SFXN1 also interacts with Cx32, a protein associated with Charcot-Marie-Tooth motor neuron disease. Since the function of sfxn-1.2 in mitochondrial transport at the molecular level is unknown, we aim to dissect the function and the molecular pathways of SFXN-1.2 in mitochondrial dynamics and its effects on worm behavior and neurodegeneration. Through cluster analysis and Kymograph assays, we observed that SFXN-1.2 is associated with significant changes in mitochondrial morphology and trafficking in neurons. Our results suggest a possible direct interaction between UNC-104 and mitochondria. The role of UNC-104 (KIF1A) in mitochondrial transport will have a strong impact because for decades it is thought that KIF5 and KIF1Bα are the only transporters of mitochondria. A crucial result is that the effect of sfxn-1.2 seems to be indeed specific for mitochondria such as this mutation did not affect the transport of SNB-1 (synaptobrevin-1), a common marker for “synaptic transport vesicles” and we provide clues that kinesin-1 and kinesin-3 may cooperate in transporting mitochondria. Our findings also suggest the relation between sfxn-1.2 and unc-104 at genetic and protein levels. Our results indicate that no genetic relationship exists between sfxn-1.2 and fusion/fission genes as well as that sfxn-1.2 does not affect mitochondrial bioenergetics. Neurodegeneration is common in Alzheimer's disease and Parkinson's disease; our data reveal possible motor neuron defects and sensory neuron defects in sfxn-1.2 mutants. Thus, these investigations will aid to develop novel drugs to target neurological diseases based on defects in mitochondrial transport.

157. The transcriptional landscape of nervous system development in both sexes of C. elegans

Haque, Rizwanul; Peedikayil Kurien, Sonu; Setty, Hagar; Salzberg, Yehuda; Stelzer, Gil; Oren-Suissa, Meital

Background: In sexually-reproducing organisms, the nervous systems of the two sexes have evolved sex-specific properties to mediate sex-specific behaviors. Little is known about the

genetic events that skew the developmental program in the two sexes to introduce sex-specific features into their nervous system. To gain insight into this question, we sought to create a comprehensive gene expression atlas for the two sexes of C. elegans across different developmental stages. Since male and hermaphrodite larvae are indistinguishable at early stages of development, we also developed a method to overcome this obstacle. **Results:** In the current study, we have refined a protocol by which large numbers of healthy males can be isolated at a very early stage with a purity of 98%. This enabled us to carry out whole-animal RNA seq studies across all developmental stages and in both sexes for the first time. Our results uncovered a pool of genes that are differentially/dimorphically expressed across the various developmental stages. Among those were many neuronally-expressed genes including transcription factors (such as DM domain and homeobox families), neuropeptides and GPCRs. We validated our results by comparing endogenous expression levels of our top hit genes in both sexes using CRISPR transcriptional SL2::GFP knock-in strains. Finally, we assigned sexually-dimorphic functions for the top male-biased gene in our dataset, the insulin-like neuropeptide INS-39. As predicted, INS-39 was significantly upregulated in males across all developmental stages and was expressed in different neurons in the two sexes. Using INS-39 CRISPR knock-out animals we characterized the involvement of INS-39 in L1 survival and thermosensation in both sexes. **Conclusions:** This study provides a rich database of dimorphic gene expression across development and will thus offer a foothold for establishing the roles of individual genes in dimorphic development. Furthermore, it highlights conserved candidate genes that may underlie the sexually-dimorphic manifestation of different human diseases.

158 Identifying promising therapeutics drugs entering the brain for genetic prion diseases in C. elegans.

BIZAT, Nicolas

Human prion diseases are fatal neurodegenerative disorders that include sporadic, infectious and genetic forms. Inherited Creutzfeldt-Jakob disease due to the E200K mutation of the prion protein-coding gene is the most common form of genetic prion diseases. The phenotype resembles that of sporadic Creutzfeldt-Jakob disease at both the clinical and pathological levels, with median disease duration of four months. To date, there is no available treatment for delaying the occurrence or slowing the progression of human prion diseases. Existing *in vivo* models do not allow high-throughput approaches that may facilitate the discovery of compounds targeting pathological assemblies of human prion protein or their effects on neuronal survival.

We generated a genetic model in the nematode *C. elegans*, which is devoid of any homolog of the prion protein, by expressing human prion protein with the E200K mutation in the mechanosensitive neuronal system.

Expression of E200K prion protein induced specific behavioural pattern and neurodegeneration of GFP-expressing mechanosensitive neurons, in addition to the formation of intraneuronal inclusions associated with the accumulation of a protease-resistant form of the prion protein. We demonstrated that this experimental system is a powerful tool to study the efficacy of anti-prion compounds on both prion-induced neurodegeneration and prion protein misfolding, moreover in a human PrP context. Within a library of 320 compounds approved for human use and crossing the blood-brain-barrier, we identified five molecules that were active against the aggregation of E200K prion protein and the neurodegeneration it induced in transgenic animals.

This model breaks a technological limitation in prion therapeutic research and provides a key tool to study the deleterious effect of misfolded prion protein in a well-described neuronal system and genetic organism model.

159 Effects of natural and synthetic catechol-O-methyl transferase inhibitors on two in vivo models of Parkinson's disease.
Parrales Macias, Gina Valeria

In Parkinson's disease (PD), the management of motor fluctuations aims at prolonging the effect of dopaminergic stimulation while reducing total levodopa (L-DOPA) load to avoid dyskinesia. Catechol-*O*-methyl transferase (COMT) inhibition reduces degradation of L-DOPA to 3-OMD and could prolong L-DOPA effectiveness by reducing both L-DOPA and dopamine metabolism. However, some COMT inhibitors like tolcapone and entacapone show hepatotoxicity and a real necessity to develop new drugs with a better toxic profile is highly desired.
By studying the chemical constituents of a plant, *M. pruriens*, several tetrahydroisoquinoline compounds (THIQ) derived from L-DOPA were characterized and tested on several L-DOPA enzymes metabolism enzymes including COMT (BioCIS). Some compounds have shown an absence of toxicity and a COMT inhibitory activity similar to tolcapone.
Firstly, we tested the COMT BioCIS inhibitors in an MPP+ *in vivo C. elegans* model, presenting partial loss of dopaminergic (DA) neurons and a dysfunctional specific dopamine-associated behavior. We evaluated the behavioral effect of COMT BioCIS inhibitors, comparing with tolcapone as a reference drug. After, we evaluated the different COMT inhibitors to observe if they could potentiate low doses of L-DOPA. Finally, we studied the most effective compound in an *in vivo* 6-OHDA rat model of Parkinson's disease.
We were able to restore the behavioral deficit in the MPP^{+}*C. elegans* model not only with L-DOPA but also with tolcapone and COMT inhibitors. COMT inhibitors could potentiate L-DOPA effect and the most effective BioCIS drug had similar results to tolcapone. The administration of the selected drug in the 6-OHDA rat model could confirm our results by potentiating the effect of L-DOPA. Our results pave the way for the use of COMT inhibitors on L-DOPA-treated parkinsonian patients.